

nature

May 20, 1976

Is the Sun being oversold?

THE United States will spend £36 million in 1976 on solar energy research and development. Japan will spend £18 million, France and West Germany £3 million each, and the European Economic Community £2 million. Meanwhile expenditure in the United Kingdom will be less than £500,000. Is that the right level, and if not, what is? The UK section of the International Solar Energy Society (ISES) has just published a major report (*Solar Energy: a UK assessment*, available from the Royal Institution, 21 Albemarle Street, London W1, 375pp, £10) which looks at prospects in the UK, attempts to predict the future for solar energy and tries to outline a research and development programme.

The Sun can be used for a variety of energy-related purposes: although heating applications with flat-plate collectors and electricity generation with solar cells are the best known, the report carefully outlines many other prospects. There is simply better building design, there are agricultural and biological applications of photosynthesis, including energy plantations and there are photochemical reactions; and within each of these areas there are many ingenious schemes, some of which work, many of which might, given appropriate investment of time, money and (dare we say it) energy.

The report is optimistic. It is supposed to identify areas particularly worthy of support, but after reading it one is left wondering whether anything is not worth supporting. Projects that are near to fruition should be encouraged; projects that are rank longshots should be encouraged; projects that are particularly suitable in Britain should be encouraged; projects with export potentials should be encouraged. Of course with nearly 40 scientists, engineers and civil servants collaborating enthusiastically in the report, it was unlikely that anything but an advance-on-all-fronts view would emerge, but even so the report does seem to suffer from a weak link in moving from scientific statements to policy recommendations. It is sometimes difficult to link the recommendations made at the end of each chapter to any very clearly enunciated argument within that chapter, and one suspects that the phrase "this technique could have export potential" has been added too often to have retained its credibility.

By the year 2020, the report argues, solar power could provide 35 million tonnes of coal equivalent of energy annually in the UK. It is almost impossible to estimate accurately what fraction of total energy consumption this might represent; it represents roughly 10% of present UK annual consumption. But elsewhere the report postulates that the contribution is 10% of 2020 needs, which is different, maybe by a factor of two. And one ever-optimistic Fleet Street correspondent persists in reading the figure as

30%. The report is not strong on its projections; it sometimes claims figures which are difficult, if not impossible, to find, but insofar as the projection of 35 million tonnes means anything, it probably implies that by 2020 up to 20% of all houses would be fitted with solar water heaters, and that there would be a significant contribution to our electricity supply from solar cells. (Solar energy could, of course, contribute in many valuable ways to isolated, local needs well before 2020.)

Do these predictions ring true? On the heating side they require, for instance, that in the next twenty years 0.5% of all present housing stock should be fitted with solar heaters each year. At a charitable £300 per installation and with the knowledge (not perhaps wide enough at present) that solar heating does not eliminate the need for a conventional heating system, one can only describe this prediction as hopeful. On the solar-cell side, the assumption has to be made that present costs of cells can be cut by a factor of a hundred to bring costs per watt into line with those prevailing in other electrical generators. The evidence for such a prospect seems tenuous in view of the lack of any spectacular progress up to the present in reducing costs. Further, the severe problem of cheap electrical storage will have to be confronted; the report rather lamely asserts that since the problem is receiving a great deal of attention and no fundamental scientific barrier is known to exist, "a breakthrough in the reasonable future may be expected". The same can presumably also be said of nuclear fusion.

And if solar cells experience all the required cost reductions, the land issue will have to be faced. To produce 10% of our 2020 total energy requirements from solar cells would require between 1 and 2% of the land area of Britain to be covered with solar farms (roads at present occupy a similar percentage). This is not a trivial fraction; the report asserts that the problem is "unlikely to be acute for many years as the use of solar energy will only develop slowly". But if and when solar farms do come, the size of the problem will be much greater than that of building super-highways through the country. To say, then, that solar energy is "particularly attractive in physical environmental terms" is simply to put off the day of reckoning.

The impression that the report leaves behind us is of a good survey of the science, but a loose job on the policy. This leaves the proposals for more national expenditure on research and development very exposed. ISES calls for £2 million in 1976, rising to £8 million by 1980 and £20 million in 1986. Very crudely, this would compare, in the early 1980s, with British plus EEC expenditure on fusion research. There is a real danger that over-optimism now may cause resistance to even a fraction of such money being made available later. □



International science and the Third World

NOTHING is ever done in this world until men are prepared to kill one another if it is not done. So said Shaw. Whether or not the United Nations offers a failsafe mechanism good enough to prove him wrong (it probably doesn't), the issues its agencies discuss receive too little attention. True, the UN may be ineffectual. It may be over-bureaucratic. But it is one of the few highly visible structures addressing itself to the "big" issues.

That, in fact, may be another of its problems. Whatever distance governments now put between themselves and those they purport to serve, it is but a miss compared to the mile between the

UN's "big" concerns and the everyday experience of the millions whose problems it wishes to solve. But there it is—recently, as the United Nations Environment Programme (see opposite) addressing itself to persistent if now less fashionable "eco-issues"; last month as the United Nations Law of the Sea Conference, tackling questions about economic zones and deep sea mining; right now, as the United Nations Conference on Trade and Development discussing the transfer of technology, commodities and debt problems.

If there is a common thread to all this, it concerns the ever more fragile relations between the developed and

underdeveloped countries. The problem of commodities, which bears directly on the world food problem (see page 181), and questions about the transfer of technology, encapsulate the principle issues.

But they all remain "macro" issues. If re-presentation in "micro" form is now necessary, the plea below, from a scientist in Lesotho, is a useful start in the area of science.

Lesotho is one of the poorest countries in the world. Poverty links the "macro" and the "micro" issues. And poverty, to quote Shaw once again, is the greatest of evils, and the worst of crimes.

Accommodating the Paradisian wilderness

Professor A. Brock of the Department of Physics at the National University of Lesotho in Southern Africa, draws attention to the position of the scientist working in the Third World

"PARADISIA and Dominatia: Science and the Developing World", the title of an article by Michael Moravcsik and John Ziman which appeared last year in *Foreign Affairs* (55, 699-724; 1975), struck so many familiar notes that, as a scientist working in a less developed country, I feel impelled to bring it to wider attention.

The problems are those of the individual scientist and of the small scientific community in a typical third world country—Paradisian; the relationship to be explored is the one between Paradisia and the powerful metropolitan country, Dominatia, in whose sphere of influence it once lay, in the context of science and technology, rather than that of politics, culture or economics. Science and technology are almost always viewed as desirable goods by Paradisians, and to this end the basic sciences are taught in the schools and universities, and modern technologies are adopted in industry and agriculture.

The picture of scientific poverty and intellectual isolation will be all too familiar to anyone who has visited or worked in Paradisia. Expatriate scientists will also know the feeling, but they usually enjoy the benefits of regular visits to Dominatia, and often have a network of contacts built up before they left for Paradisia. The Paradisian scientist, on the other hand, has fewer opportunities of this type because he cannot qualify for "home" leave, and his contacts in Dominatia derive mainly from his days as a research student rather than from a period of mature scientific practice. For him the keyword is isolation.

The concern here derives from the view that science flourishes through the efforts of individual scientists, but only in the context of an active scientific community which provides many channels (both official and unofficial) for encouragement, criticism, review, stimulation, interaction and publication. This is the rich soil in which science grows. But in Paradisia the scientific climate is semi-arid and the soils desert-like.

Take, for example, the keen Dominatian scientist on secondment to Paradisia, prepared to improvise in the absence of technical facilities, but handicapped beyond measure by the arrival of *Phys. Rev.* a year late and the absence of colleagues to discuss his work with. His productivity, it is suggested, will rapidly decay under these circumstances. A more personal example: as a palaeomagnetist, I have been fortunate to find my material in Paradisia—fresh Precambrian lavas, or richly fossiliferous Plio-Pleistocene lake sediments. But my results take shape in correspondence with colleagues in Canberra, Stanford, Berkeley or Leeds, rather than over the tea table in my own laboratory. How much more difficult for a Paradisian scientist forced by circumstances to rely almost exclusively upon his own resources. And what a triumph for those who manage to produce substantial scientific work.

The task of helping Paradisian scientists working under such circumstances is more difficult than that presented by more normal aid programmes, in which buildings or equipment or funds are provided for some specific purpose, such as a soil science laboratory, or a

natural resource survey, or a feasibility study. The task is that of trying to create and maintain indigenous scientific activity in Paradisia. The suggestion is that the world scientific community bears some responsibility towards its less fortunate members in Paradisia, by extending to them some of the advantages that are taken for granted in Dominatia. Lavish funding *per se* is not envisaged here, although it is worth remarking that even the most heavily "squeezed" research budget in the UK would look lavish to a Paradisian.

It is the opportunity for full and free participation in the scientific processes of discussion and exchange, the feeling of "belonging", that Paradisians particularly lack. Topics such as the provision and distribution of journals, the suitable placement of research students, attendance at conferences, the exchange of staff, academic visits, are suggested as areas in which careful attention to the needs of Paradisia would pay dividends. The development of an active lively scientific community is a lengthy process, and cannot be the object of any single aid programme.

One suggestion, though, is a device for opening a range of scientific appointments in Paradisia to young scientists from Dominatia as a normal stage in a research career. Another is that free copies of the major scientific journals be sent to all accredited scientific institutions in the less developed countries. What a blessing this would be to "out of touch" scientific workers. These suggestions apart, however, the concern should be to alert the world scientific community to the general plight of the small fraction of its members in Paradisia, and to suggest that there is much that can be done at the individual as well as the institutional level to help. □

Two steps forward, one step back

The United Nations Environment Programme seems to be faltering. **Richard Sandbrook** reports on the efforts to deal with environmental problems on a global basis

THE United Nations Environment Programme (UNEP) has been having an uncomfortable time of late. Not only are funding problems now severely hampering the agency; there also appears to be increasing dissent about the work programme, both within and without the Secretariat. Nothing new in one sense for the UN; but at a time when many states are taking a new look at the UN in general following the recent "group of experts" report on its structure, and when its major donor, the United States, is severely pruning budgets, uncertainty in UNEP is not going to help its causes.

Initially UNEP was set up to carry through the 109 recommendations that came out of the UN Stockholm Conference on the Human Environment held in 1972. It was structured to consist of a Programme, which is the outline of the projects being tackled by UNEP, other UN agencies, national governments or non-governmental organisations; the Environment Fund, into which states make voluntary contributions; the Secretariat, consisting of 100 professional staff; and a Governing Council, composed of 58 UN nations, which meets annually to review the programme and state of the fund. In addition, the executive director of UNEP chairs the Environment Coordination Board, which includes the heads of all other agencies in the UN family.

The fund started out with pledged contributions of \$110 million to carry it through its initial five years, and the energetic services of Maurice Strong, its first executive director. Strong had left his mark by the time he departed in January. He had tried hard to dispense with many of the usual UN non-senses that slow up the whole business of running the existing UN agencies. Contracts were handed out in an almost commercial manner—rather as if a new research and development programme was in the making—and initially he circumvented the delaying process of UN recruitment by appointing consultants. The relationships between UNEP and non-governmental organisations, governments and other specialised agencies was—and still is—fluid and open. The programme itself was neatly compartmentalised into priority levels and clear-cut objectives.

But UNEP had its fair share of teething problems too. Nairobi, the home of the programme, is not as accessible as the more "traditional" Geneva and New York, a particular disadvantage since the whole venture was set up partially to get existing outfits, such as the FAO, UNESCO and WHO, to cooperate together through the newly created Environment Coordination Board. Mr Strong and his team spent many months in the first three years commuting between the world's capitals for planning and promotional meetings. Back in Nairobi permanent staff recruitment was very slow, and the planning of programme projects became delayed. By the 1975 governing council meeting the fund was carrying forward large surpluses and certain states were complaining that not enough was being spent; UNEP had too much government money in its bank earning interest. Ironically this now forms the central excuse of the US Government for delaying their "promised" contributions (initially a 40% slice of the first five-year \$110m).

Changing attitudes

Attitudes to the "environment problem" also began to change. Strong was above all a superb salesman who generated great interest and concern before Stockholm and immediately after it. In the early 1970s "the environment" was an important issue for western politicians. Now, some four years on, the environment issue is clearly not as politically pressing as it was; and, besides, many governments would argue that national pollution control is advancing rapidly. Initially the less developed world was very suspicious of the whole affair. Strong's answer was to promote "eco-development"—or "environment and development"—and a challenging declaration that united these themes was developed (the COCOYOC Declaration 1974). Gradually a constructive relationship built up between UNEP and the doubting Third World nations.

UNEP, however, has never been seen as a route for aid or technical assistance in the eyes of the major western donor countries—it is much more a "catalyst" (the most used word by delegates to the recent 1976 governing council) for sound environmental management. The idea of UNEP growing into an enlightened development agency with funding capabilities

is certainly not acceptable. All this leaves the impression that the idea was potentially good; but for some the fashion is now passing, while for others UNEP's objectives are becoming less relevant. The current acting executive director, Dr Tolba, is nobly conducting a holding operation, but eventually the big issues will have to be resolved.

The first and major issue to be sorted out is the leadership question. Dr Tolba, an Egyptian, is due to leave this year; he could emerge as the new man from the horsetrading that goes on in New York, but the OECD countries and the South Americans may put up a challenge. Then there is the problem of replacing before the end of the year Mr Steadman, who runs the fund, and Mr Munro, who directs the programme. Both are available for office for only a short period. UNEP has always been beset with changes at the top, and this has created uncertainty all the way down.

Financial problems

The second issue is the state of the fund. The long lead time on project approval in the formative years has pushed the peak cash needs of UNEP toward the end of its first quinquennium. The cash requirements double between 1975 and 1976 to \$40 million and then slowly climb to an annual requirement of \$55 million by 1979. (It is worth noting that the US Federal outlay in 1976 on pollution control and abatement was \$683 million for research and development activities alone.) Currently, the agency predicts a shortfall of some \$10 million to \$12 million a year from 1977 onwards. This figure is based on the revised pledges known at March 15 this year, some of which are well below those originally given when the agency was established. Commenting at the governing council in April, Tolba said that only \$41 million of the pledged \$109 million had so far been paid up, and some of that was in non-convertible currency from the USSR. The US was singled out as the major turncoat. Special visits to Moscow and probably the US are planned, but matters look bad: some 42 projects, Tolba reported, had already been delayed because of the cash crisis. Things could conceivably become a little easier for the agency after the US elections.

The third problem concerns the dream of Stockholm itself: how effective it will be in practical terms will in turn affect the long-term support UNEP gets. To date UNEP has successfully translated a few good ideas, such as that of the FAO for a Mediterranean pollution convention, into actual events (Barcelona, March 1976). They have also been closely involved in planning and funding the UN Water

Conference (March 7, 1977, Argentina), the UN Deserts Conference (August 29, 1977, Nairobi), and the imminent UN Human Settlements Conference (May 31 to June 14, Vancouver). Much credit must go to UNEP for causing the latter two events to happen at all. All these conferences will help establish priorities and routes for aid and technical assistance in the coming years on pressing development fronts.

Tasks remain

But UN conferences can only assist in the evolution of "global environmental management"; all too often they seem to result in little more than high sounding clichés. Substantive tasks remain: the world's disappearing rainforests, the protection of the ever-growing number of threatened species, the chronically over-fished marine resources, rising levels of air and water pollution, to name a few. Whether or not resolutions which "urge" and reports which "chastise" are enough, UNEP is not in the final analysis going to be able to do very much on its own. It does not have the necessary expertise or financial base, and governments are not going to provide either. Division Three of UNEP, which handles environment and development issues, for example, recently suggested that a series of Third World alternative energy promotion centres be set up to provide practical and working alternatives to the current extremes of forest removal for firewood on the one hand and capital intensive, energy generating technologies on the other. Some delegations to the governing council clearly saw this as a subtle route to aid. One centre is enough for all to learn by, it was argued, and they were reduced in number to one. The

idea of doing it regionally was ruled out.

Earthwatch is, in fact, the only significant functional task that UNEP is involved in apart from its conference support work. The remainder consists of overscanning some 150 projects to be carried out by other UN agencies, governments and institutions. These are organised into some eight priority areas, as follows: Human settlements and human health (1976 Budget \$6.3 million), Ecosystems research (1976 \$8.19 million), Environment and development (\$3.6 million), Oceans (\$3.6 million), Energy (\$550,000), Natural disasters (\$200,000), Environmental management (\$450,000), Environmental law (\$300,000) and support functions (\$5.91 million). Earthwatch takes \$3.4 million this year.

Earthwatch consists of the International Referral System (IRS), the Global Environmental Monitoring Systems (GEMS), the International Register of Potentially Toxic Chemicals (IRPTC) and the Study of Outer Limits (climate change, risks to the ozone layer, increase in bioproductivity and weather modification). The aim of IRS is to have a global network of contacts in operation—a kind of environmental "yellow pages"—based on a data bank in Geneva. The system will consist of over 50 data focal points (to date only one is operational in the US; the Department of the Environment in Britain hopes to participate fully later this year). These feed in the basic data they have on the activities of various government departments, institutions and individuals in the environmental field. GEMS is trying to monitor key environmental variables on a global basis and thus lead to an assessment of the state of the environment in seven

distinct areas. These include atmosphere monitoring in relation to climate, ocean monitoring, the depletion of renewable resources, natural disasters and human health. Throughout there is much emphasis on the concept of baseline studies.

Coordination only

UNEP is the first to point out that much of this monitoring is already done by other agencies and that its role is one of coordination only. There is, for example, UNESCO's Man and the Biosphere Programme; the work of the World Meteorological Organisation on climate; the FAO's work on land use, fisheries and soil structure; and, of course, many governments have their own monitoring programmes. It is hoped that variables will be monitored around the globe in a uniform and comparable way, and the data collated into central locations. But the problems in practice are going to be considerable. So-called baseline studies in the marine environment, for example, are notoriously difficult to carry out, and to many are of dubious scientific value anyway. To determine the quantities of various pollutant involved from atmospheric and land sources and their effect on the biological system is an almost impossible task; the state of the initial biological system is usually not known, is probably inherently variable, and the pathways of the pollutant far from clear.

It is thus apparent that UNEP structures are again in a state of wait and see. Until the financial and leadership questions are cleared up its future direction and style are unclear. At present, it is prevented from being the radical outfit that the Third World want by the priorities of its powerful western backers. Any confrontation with the real forces that lead to environmental degradation — inequality between nations and over-indulgent living as Mr Strong once put it—must wait. As the executive director stated in April, four years after Stockholm every country has come to grips with the fact that sustained development and meaningful growth were impossible without a commitment to preserve the environment and promote the rational use of resources. But if the Secretariat has adopted the principles of the brave new world, its major funding source has not. And yet, leaving the development issue aside, there is a clear need for UNEP simply to coordinate the significant effort being made toward the protection of the biosphere at a national, regional and international level. It has gone a long way in convincing governments and international agencies that this should be done. It would be a great pity if it were now denied the wherewithal to do it. □



More than just an environmental problem: the litter and decay of a forgotten community (WHO photo)

When enough is not enough

Howard Rush, Pauline Marstrand, John Gribbin and Gordon MacKerron look at what is sometimes called the world food paradox

WITHIN the past few months a series of publications* has again focused attention on the complex of problems underlying the "world food crisis". These analyses aim at different audiences, but all have a message in common: our inability to feed the world's population is not due to physical factors placing limitations on total world food production. They also recognise that the causal factors in world hunger and malnutrition are the enormous economic, social and political problems associated with poverty.

The key physical factor, of course, is availability of land. Most recent estimates are based on calculations published in 1967 by the US President's Science Advisory Committee (PSAC), suggesting that the maximum area which would be cropped is 6,600 million hectares, of which about half has been cultivated at some time and about 1,400 million hectares harvested in any one year. Although other estimates—such as those by FAO and the US Department of Agriculture—have produced a broad spread of figures, the PSAC estimates are roughly in the middle of the range, and all investigators agree that only about half of the potential area is now used. The estimates differ partly because of different assumptions about the feasibility of obtaining water, but they all show that apart from competing uses of land, existing world food production could be roughly doubled without improved technology.

Lester Brown and others have postulated that increasing costs of development will preclude utilisation of much of this land—an assumption also built into the *Limits to Growth* model—but there is room to query this assumption. Although it is true that the tendency is for the range of available technologies to move towards the very capital intensive (and expensive) end of the spectrum, in principle developing countries can still select from alternatives ranging down to such labour intensive projects as the human muscle used to build the Yangtse dam. There is certainly room here for further

debate, partly because future cost assumptions depend so heavily on the nature and pace of future technological change.

Using the PSAC estimates, the maximum population supportable has been taken as 7,200 million at existing standards of nutrition, or 157,000 million on a 1967 "Japanese" diet. Although it is commonly stated that 400–500 million people are starving in the world today, it seems unlikely on the basis of WHO estimates that more than 60–70 million are currently so severely undernourished that they are using bodily protein reserves (muscles, including heart muscle) as a source of metabolic energy.

The important factors

This represents less than 2% of the present world population, and is probably a smaller proportion than ever before, but it is nothing to be proud of; the efforts made over the past three decades to improve food supply to the hungry have been successful in part and are worth sustaining. But if physical factors are not critical in determining the availability of food to the world's population now and in the immediate future, what are the important factors—and are they really so enormous as to provide insurmountable difficulties?

The key to the world food "problem" is now seen to be poverty, not population. Poverty within nations means that poor people receive a smaller share of the food available nationally, while poverty between nations means that the poorer nations have less food per head to share in the first place. And the inequalities are greatest in the poorest nations, so that the smallest slices of the cake are themselves divided the most unfairly.

Between 1951 and 1971 the global production of cereals more than doubled, while the world's population "only" increased by 50%. More than half of the increase in food, however,

was absorbed by the richest 30% of mankind, so that the extra food was shared almost equally between 1,000 million people in the developed countries (DCs) and the 2,500 million people in the less developed countries (LDCs). This pattern emphasises that the food is there—or can be produced—for those who can afford it. It is because they are poor that the hungry—whether they be nations or individuals—are unable to buy sufficient food, or to develop their farming sufficiently to provide for their own needs. Under the present world economic system, their hunger, as such, is largely irrelevant; "demand", in the food market as in other markets, means ability (or willingness) to pay. As in other free markets, the rich have a dominant influence: their power in the market distorts demand patterns, and allows them to obtain the greatest benefits for themselves. One important result is that in the poor nations—as well as the rich—increasing demand for meat prevents the most effective use of available land by diverting large areas of cereal from human to (inefficient) animal consumption.

It was a reduction in this market demand that led to a reduction in planted cereal acreage in the developed countries during the 1960s, at a time when there was clearly an increasing need for food among the hungry poor who had no financial access to, or influence on, the market. Between 1960 and 1970 the LDCs were increasing food production at least as fast as the developed countries, but the increasing market demand for "luxury" food—beef, dairy produce and so on—forced prices upwards, including the prices of cereals used for feeding animals as well as people. Together with the emphasis at government level in many LDCs on cash crops that could be sold to developed countries to boost exports (coffee, sugar and so on) this had serious effects on the poor in the LDCs, where even if luxury foods were being exported essential foods had still to be imported in bulk (as represented in Table 1).

The shortages of the early 1970s and high prices have been blamed on many natural factors, not least the weather

Table 1 Changing Patterns in the Grain Trade (million metric tons)

Region	1934–38	1948–52	1960	1966	1973*
North America	+5	+23	+39	+59	+91
Latin America	+9	+1	**	+5	–3
West Europe	–24	–22	–25	+5	–3
East Europe & USSR	+5	**	0	–4	–27
Africa	+1	0	–2	–7	–5
Asia	+2	–6	–17	–34	–43
Australia, New Zealand	+3	+3	+6	+8	+6

Plus sign indicates net exports; minus sign net imports

*Estimated **Not available

Source: *Financial Times*

*Future World Trends, UK Cabinet Office (April 1976); *The World Food Crisis and Third World Development: Implications for UK Policy*, UK Select Committee on Overseas Development (February 1976); *Profits of Doom*, C. Robbins and Javed Ansair (War on Want, January 1976); *World Food: A Political Task*, Howard Wagstaff (Fabian Research Series 326).

The authors are all at the Science Policy Research Unit, University of Sussex.

(which, in fact, was not so much bad as simply worse than the previous, exceptionally good, years of the 1960s). But these shortages, although real, were not physically necessary; they resulted from the control of the market by producers in the developed countries.

LDCs can only pay for the food they import with the foreign exchange earned by their exports; ironically, agricultural produce makes up three-quarters of those exports, but the LDCs' share of the world market in these products is falling as the developed countries produce more and increase their domination of the market. According to War on Want, "In 1966, prices for exports from the DCs were 13% higher than in 1958 while prices for exports from the LDCs fell by 11% . . . more and more has to be exported to buy the same goods from the DCs". The resulting imbalance, with the developing nations' debts to foreign countries increasing substantially faster than their GNP, means that in the majority of cases the gap between debts contracted and the means of repaying them is widening every year. As this gap increases so do the inequalities between developed and developing nations.

Internal factors

The responsibility for this, however, does not lie solely with the developed nations' control over world markets. Their market control does place the poor of developing nations at a severe

disadvantage, but the inequalities are heavily reinforced by agricultural policies and local price factors within the developing nations themselves, where agriculture accounts for more than a third of total production and rural regions provide about three-quarters of the total population.

These regions and most of their impoverished population have little say or control over their economy. The majority are landless or own less than a single hectare of land. What little agricultural policy there is in many LDCs, including investment programmes, is heavily biased in favour of the prosperous minority. The result of this imbalance is clearly seen in the example of India, where 7.7% of the population own more than 50% of the head. More often than not the situation is made worse by national development policies which stress industrialisation of urban areas dependent on the agricultural sector for resources.

As Keith Griffin has pointed out, the outstanding feature of the agricultural sector in almost all LDCs is the bias in access to factors of production (that is, land, credit, water, fertilizers, technical knowledge, etc.) towards the prosperous landowners. Government policies supported by the economic and political influence of this minority virtually control the market structure and allocation of resources—thus assuring no shift in the *status quo* detrimental to their interests.

Technical change, once heralded in the form of the "green revolution" as

the solution to man's hunger, becomes a further aid to the rich because of their dominance of the world system. Technical change which relies on easy access to credit and often substantial inputs of water, fertilizer and pesticides, may well increase production—but only for the minority of landowners in whose favour the market is biased. Any innovation under the control of the *status quo* tends to strengthen the *status quo*. Agricultural innovation has become widespread, but the result has not only been increases in productivity but also increases in inequality; the rich get richer, but the poor get poorer.

In recent years the world's food supply has increased faster than the rate of population growth. As suggested by nearly all recent analysis, physical factors are not critical to feeding the world's population. But the fact that fewer people need face starvation than previously believed does not reduce the seriousness of the difficulties which remain: the problems are now recognised as those of poverty and politics. It might seem that these are no less insurmountable than the physical restrictions often assumed—although we could feed a much larger population, it may require a social revolution to remove the inequalities at the root of poverty. As the UK Select Committee on Overseas Development stressed in its report, "the main answer to the world food problem is to give those who are hungry the means to feed themselves, or the income to buy food". □

USA

Antidisestablishmentarianism

Arrangements by which the US President receives advice on science matters have now been institutionalised. Colin Norman reports from Washington.

WITH storm clouds hovering overhead, and with many elder statesmen of the scientific community in attendance, President Ford last week held a small ceremony in the White House rose garden to mark the signing into law of a bill re-establishing a science policy office in the White House.

The event was pure ceremony. Contrary to rumours flying around beforehand, Mr Ford did not nominate anybody to head the office—the director will also be the President's Science Adviser—nor did he indicate how he intends to use the office when it is eventually set up. Instead, he simply took the opportunity to make a few obligatory remarks about the importance of science and technology in

helping to meet "the challenges and opportunities which lie ahead for this nation and the world", and signed the bill with "great pleasure". Fortunately, the rain held off.

The ceremony nevertheless marked an important event in the annals of science policy. The bill, which had been championed by numerous prominent scientists and also by Vice-President Nelson Rockefeller, essentially reinstates the science policy apparatus dismantled three years ago by Mr Nixon. Moreover, since the office has now been established by an Act of Congress—rather than an act of Presidential pique—to remove it.

Though scientists, of course, played a prominent role in shaping national policy during the Second World War, they didn't have a permanent place in the White House until 1957, when President Eisenhower acquired a full-time science adviser during the post-Sputnik panic which swept the

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President Ford signs the science policy bill, watched by (left) Senator Frank Moss, chairman of the Senate Space Committee, and Representative Olin Teague, chairman of the House Committee on Science and Technology.

N.E.W.S. photo

country. The adviser was made chairman of the President's Science Advisory Committee, a panel of scientists drawn from academie and industry. President Kennedy established the Office of Science and Technology (OST)

THE Senate last week followed the lead of the House of Representatives by voting to cut off federal support for a controversial research project designed to assess the effects, if any, of marijuana on human sexual behaviour. The Senate's action will ensure that the project will be aborted, and thus for the first time Congress has reached deep into the scientific peer review process and cut off a research project for political reasons.

The project, a \$121,000 two-year investigation which was to have been conducted by Dr Harris Rubin at Southern Illinois University, had been approved as scientifically meritorious by a committee of behavioural scientists, endorsed by a top level government advisory committee, and supported by a panel consisting of the federal government's most senior health officials. Congress voted to stop the project, however, because some powerful members have labelled it morally unacceptable and scientifically trivial.

The Senate, at least, held a short debate on the matter before deciding to shut the project off. Senator William D. Hathaway, Democrat from Maine, offered an amendment to restore the funds, arguing that "the real issue is the integrity of a carefully constructed government-wide mechanism for awarding research grants and contracts". He argued that by voting to deny the funds, "possibly the least expert group of federal employees to gather in one building—the US Congress—seems now to have taken upon itself the role of grand inquisitor with regard to scientific research". Hathaway's amendment was easily defeated, however, on a voice vote.

It's not too surprising that, once the issue was raised, Congress should vote to eliminate Harris's project. It is, after all, an election year, and Congressmen would be reluctant to defend a vote in favour of spending tax payers' money on a project involving marijuana and sex. But, as the Federation of American Scientists

(FAS) noted in a statement last week, the votes set a worrying precedent. "Every controversial project that finds its anti-champion can now be expected to be raised" in Congress, FAS suggests.



● After months of debate by several committees of scientists and a week of conflicting rumours, the Food and Drug Administration (FDA) formally announced last week that it has no intention of lifting its controversial ban on the artificial sweetener cyclamate. Citing a number of "unresolved safety questions", FDA Commissioner Dr Alexander Schmidt said that he has asked Abbott Laboratories, the manufacturer of cyclamate, to withdraw its petition seeking to restore the sweetener to the market. If the petition is not withdrawn, Schmidt said he would simply reject it.

Cyclamate was banned in 1969, on the basis of studies which suggested that it increased the incidence of bladder tumours in rats when fed to them in high doses over prolonged periods. The FDA ban initiated a chain of similar actions in other countries.

The FDA's decision to keep cyclamate off the market is, however, based more on questions of general toxicity than on the shaky evidence that cyclamate is a carcinogen. A panel of scientific experts, assembled by the National Cancer Institute, reported last February that "although the

present evidence does not establish the carcinogenicity of cyclamate", a number of studies have raised worrying questions. In short, the evidence for carcinogenicity is equivocal.

Schmidt said last week that he is worried about "unanswered questions" concerning cyclamate's potential for causing damage to reproductive organs, causing chromosomal damage, and elevating blood pressure.

A spokesman for Abbott has said that it is unlikely that more safety tests will be conducted to resolve those questions, and it is therefore unlikely that Abbott will renew its petition. FDA's decision thus could be the final word in the long battle over the sweetener.

● Mr Jimmy Carter, the leading candidate to be the Democratic Party's Presidential nominee, last week called for a voluntary, worldwide moratorium on the sale of uranium enrichment and nuclear fuel reprocessing plants, including those sales already negotiated. Speaking at a conference at the United Nations Centre, Carter said that the threat of nuclear weapons proliferation from such sales is too great for "business as usual". He called for an un-sponsored world energy conference to discuss worldwide energy problems, and he said that "there is a moral imperative that demands a worldwide effort to ensure that if we travel down the nuclear road we do so with our eyes open".

The nuclear powers, he suggested, should provide more leadership in preventing the spread of nuclear weapons. For a start, he suggested that the United States and the Soviet Union should agree on a five-year moratorium on weapons tests, during which they should negotiate a complete nuclear test ban. The recently negotiated threshold test ban, he said, is "wholly inadequate".

As for the US nuclear power programme, Carter suggested that it should be kept to "the minimum necessary to meet our needs", and it should have to meet "much stronger safety standards".

five years later, as a small White House operation headed by his science adviser, and that apparatus remained intact until 1973. President Nixon then suddenly scrapped it, largely because he disagreed with much of the advice it was offering him, particularly on such matters as the need for an anti-ballistic missile system and the SST programme. When Mr Nixon abolished OST, he gave the head of the National Science Foundation the extra job of being science adviser to the White House.

The bill signed by Mr Ford last

week will establish a small Office of Science and Technology Policy (OSTP) to advise the President and other White House bodies on matters involving science and technology. It also establishes a committee of scientists to conduct a two-year review of the federal government's science and technology programmes, after which the committee can be kept in business as an advisory unit if the President so desires. White House officials say that President Ford hopes soon to nominate somebody as his science adviser, but it

will take several weeks to get the office under way.

It should be noted that the establishment of the office couldn't have come at a much worse time. Having suffered a series of staggering defeats in primary elections these past two weeks, Mr Ford's tenure in the White House must be considered precarious, at best. Thus, with a change of Administration at least on the cards a few months after OSTP is established, nothing dramatic should be expected from the office for some time. □

CANADA

Spraying controversy revived

A link is suspected between the use of certain tree sprays and the condition known as Reye's syndrome. David Spurgeon reports from Ottawa

THE spraying of forests in the Maritime provinces to prevent infestation by the spruce budworm has created a sharper controversy than ever this spring because of the deaths of five children from a disease suspected of being linked to the insecticides used. It is not the first time arguments have arisen over the spraying programme: DDT was used as an insecticide in the 1950s and 1960s until it was found to have caused severe fish kills in surface waters. But it is the first time human deaths have been linked to the programme.

A number of children, mostly from areas that have been heavily sprayed in the past, have died from Reye's syndrome, a condition first described in 1963 following the US Air Force's aerial defoliation in Vietnam and Thailand. The disease causes increased susceptibility to viral infection, and it is the spray dispersal emulsifiers which are suspect; the sprays used are Fenitrothion and Phosphamidon.

The current budworm outbreak, which now covers about a third of the province of Nova Scotia, was first reported in 1969. The budworm (*Choristoneura fumiferana*) is a moth that lays its eggs in firs and spruces in July. The eggs hatch in about 10 days, and the larvae spread through the trees.

They remain dormant for the winter, emerging in late April and early May to begin feeding on new shoots. In June they enter the pupal stage, and the moths which emerge in June or July can defoliate and kill trees to the extent of causing their death.

Massive increases in population can occur when there is a large incidence of host trees and favourable weather. This has happened in the Maritime provinces six times since the early 1700s. Predictions for this year were bad from the point of view of the forest industries, particularly in Cape Breton Island, so Nova Scotia Pulp Ltd and Nova Scotia Forest Industries—a branch of Stora Kopparbergs Bergslags AB of Sweden—applied to the Nova Scotia government for permission to spray with Fenitrothion or Phosphamidon.

The Nova Scotia department of lands and forests recommended against it, but the Cabinet gave approval in February. Seven weeks later, on April 2, Cabinet approval was withdrawn as a result of a story that appeared first in the *Cape Breton Post* and subsequently in other newspapers. The story summarised the work of a research team at Dalhousie University begun in 1974 which indicated that a link was possible between the spraying programme and the deaths from Reye's syndrome. It was based on material given to the *Post's* editor by a PhD student who was a member of an environmentalist group.

The research group had published a

preliminary report on their work in *The Lancet* in 1974, and has a further paper in press now. Its members applied spray chemicals topically to the abdomens of about 13,000 mice in the same concentrations used in the spraying programme, and then exposed the animals to a virus. They found that the mice died, not as a result of the virus or of toxin but of some third entity. At the time they published in 1974, they had applied only the insecticides as sprayed, to the animals. Later they purchased the carrier substances separately and concluded that one of the emulsifiers used in the sprays was probably the substance linked to Reye's syndrome.

Dr J. F. S. Crocker, one of the research group and an assistant professor of paediatrics, emphasises that the research has been strictly with animals and indicates only that a link between the spraying programme and the deaths from Reye's syndrome "is possible theoretically." The group is now planning *in vitro* experiments and work with primates, and has proposed identifying chemicals in the tissues of children struck by Reye's syndrome—if possible before they succumb. Dr Crocker himself finds it extraordinary that so little research has been done in this field. He says that if the companies that do the spraying make up their own spray formulation, they do not even require a licence for the emulsifiers, whereas the spray mixture must be licenced if it is packaged as a ready-made formulation. Yet the chemical structure of the emulsifiers is unknown—and of course the effects on humans are unknown.

The budworm problem has been worst in New Brunswick in past years, and has only recently spread to Nova Scotia. No spraying has actually been carried out in Nova Scotia, but in New Brunswick it has always been carried out by Forest Protection Ltd, a Crown corporation representing the provinces and its major pulp and paper industries. Decisions about whether to spray or not in a given area have been made by the corporation's board of directors and approved by federal and provincial governments. Costs have been shared equally by the federal government, provincial government and industry. Fenitrothion has been the chief insecticide used since 1968: it is claimed to be non-persistent, non-cumulative and not subject to concentration in the food chain. There is also evidence it is less harmful to fish than is DDT.

This year's spraying programme in New Brunswick will cover 10.2 million acres. The federal government has opted out of the programme, but the federal department of regional eco-

Canadian cutbacks

THE Royal Society of Canada has joined in the general clamour of protest by Canadian scientists against a federal freeze on research funds. The Prime Minister, M. Pierre Trudeau, recently received a brief signed by W. Bennett Lewis, president of the council of the Society's Academy of Science, which said the 1975-76 level of federal support for government laboratories was only 83.3% of the 1969 level in terms of the fraction of federal budgetary expenditures. Research in Canadian universities is in an even worse position, with support in 1975 reduced to 67% of the 1969 level—and although the decline was arrested last year, the freezing of grants this year will cause support to decline to about 60% of the 1969 level.

Although it was recognised in 1969

that government support of industrial research and development was already too small, it is now even smaller, the brief said. In terms of the fraction of federal budgetary expenditures it had fallen by 1975 to 68% of the 1969 level. To make matters worse, the Department of Industry, Trade and Commerce had abolished one of its aid-to-industry research and development programmes and had cut back another.

Tables and graphs accompanying the brief showed that Canada was alone among advanced nations in its reductions in support for science and technology. Organisation in other industrialised countries with functions similar to those of the National Research Council of Canada (supporting in-house research and development and university grants and scholarships) had been treated almost as well, and in some cases better, than they were in 1969.

conomic expansion will contribute. As a result of the controversy in Nova Scotia, questions have been asked in the New Brunswick house about the advisability of carrying on with the programme. In an article to be published in the June issue of *Science Forum*, Neal Benneworth and Chris Bailey contend that spraying should be prohibited because it is "an energy-intensive attempt to bend nature to conform with the forest industry's narrow concepts of how a forest should

behave".

They say the operation only increases in the long term the chances of occurrence of what it was originally intended to prevent, because preservation of the host trees by spraying "simply improves the environment for the budworm and increases the likelihood of further population expansion." This is so because the forest and the budworm are a self-regulatory system: the budworm destroys the forest and ensures a new development of host species favourable

for future budworm generations. The present composition of the forest, they say, has evolved as a result of budworm predation removing mature balsam fir preferentially, which allows less competitive species of trees to survive. Budworm spraying merely protects the host trees, which are economically significant to the forest industry, and this in turn improves the environment for the budworm, thus increasing the likelihood of further population expansion. □

USSR

ONE of the obstacles to communication between Soviet geologists and their foreign colleagues in recent years has been the unwillingness of Soviet geologists officially to admit the possibility of continental drift. This block was not due to any political considerations—unlike those imposed in Stalin's time on genetics and cybernetics—but seems to have been due to personal opposition from certain leading geologists, notably Professor V. V. Belousov of the Institute of Earth Physics in Moscow. Recently there has been a discernible relaxation in this attitude: the popular science magazine *Priroda* has carried an article dealing with this "hypothesis", and even Professor Belousov himself has somewhat half-heartedly discussed the subject in his latest book.

Now, however, a TASS radio bulletin, transmitted in English and hence clearly for international consumption, has given even greater approval to this concept, stating that Australian rock samples, collected by M. Ravich of the Institute of Arctic Geology in Leningrad, have proved to be "completely identical" to samples from the Antarctic opposite Australia, thereby giving added proof of the "so-called theory of Gondwanaland". According to Professor Ravich, the samples match not only in chemical composition and age (2,000 million years), but also in the size of the deposits, and give credence to predictions of mineral wealth in the Antarctic similar to deposits in Australia, South Africa and South America. A number of Soviet geologists have privately supported the concept of continental drift for some time. The TASS broadcast indicates a possible reversal of the official view of the Soviet geological establishment.

● The chance a private individual in the USSR has to participate in a major scientific research project nowadays is extremely limited. But it was granted to the citizens of Byelorussia, the Baltic States and the Leningrad-Novgorod region of the

Russian Republic by the flight, on February 11 this year, of what has been named the "Baltic bolide".

This large meteorite, which appeared in the twilight over the Gulf of Finland, crossed the gulf moving in the general direction of Moscow before burning up. Its flight time of



more than 100 seconds, instead of the normal one second or so, is a record for any meteor, and is attributed to a velocity comparable with that of the Earth and a trajectory virtually tangential to it. Before the bolide finally broke up with a fine display of natural pyrotechnics, some 600 people following it along its path had observed it closely enough for their letters and sketches to help astronomers at the Pulkovo observatory plot its course. According to V. Kratt, the observatory's Director, the bolide ceased glowing only at a height of 25 km, and there is reason to hope that some fragments may have penetrated the atmosphere to ground level. The Soviet Academy of Sciences has therefore appealed to all persons living along the route to survey the area for fragments.

● The State Committee for Science and Technology of the USSR's Council of Ministers has signed an agree-

ment on technical cooperation with British Petroleum, to run for an initial period of five years. It provides for broad-based reciprocal scientific and technical cooperation on a long term basis, and is the result of some two years of negotiation, since Academician Vladimir A. Kirillin, the committee's Chairman, visited Britain in 1974.

The agreement envisages exchanges of scientific and technical information and of specialist staff, joint research and development, the organisation of symposia on topics of mutual interest, and the joint implementation of research programmes and projects—on the production of unicellular proteins from petroleum wastes, for example. The State Committee has shown considerable interest in purchasing the expertise for deep-water drilling developed by BP in connection with the Forties field in the North Sea.

One new Soviet development in anti-pollution measures, however, is unlikely to form part of any exchange agreement. According to Moscow radio a new agent for cleaning up oil spills in the Caspian has been discovered: the cockle *Cerastoderma*, which, although only some 2.5 cm long, filters up to 15 litres of water through its body in its feeding process. Any oil contained in this water is coated with slime and eliminated, forming a sludge which falls to the sea-bed, where, it is thought, it is later rendered harmless biologically.

● The latest volume of the Large Soviet Encyclopedia carries a brief, 75-word biography of Andrei Sakharov, complete with photograph, describing him as a "Soviet physicist and Academician" and recording that he was thrice awarded the title of Hero of Socialist Labour (the highest Soviet civilian award). Sakharov's human rights work is nowhere mentioned: his recent activity is dismissed with the remark that "in recent years he departed from scientific activity". Sakharov himself commented that he was "very pleased" with the entry.

Vera Rich

UK ENERGY

BRITAIN'S "Save It" energy conservation campaign clearly doesn't apply to the increasingly protracted debates on the subject itself. Not that the energy spent discussing the issues is entirely wasted, even though it certainly looked that way three weeks ago when the latest rush of events was about to start.

What research and development was in hand, a backbench MP asked, and what new technology developed, so that alternative sources of energy could, if economic, be rapidly exploited? Mr Anthony Wedgwood Benn, Secretary of State for Energy and Britain's principal advocate of open government, was simply uninformative:

Considerable research and development is in hand on indigenous raw materials which are or might be sources of energy. Much of this work is carried out by the nationalised energy industries, the United Kingdom Atomic Energy Authority (UKAEA), the nuclear industry and the oil companies. In the field of new non-nuclear energy sources, my Energy Technology Support Unit has carried out assessment studies. Research and development programmes are being drawn up for those areas which show most promise of making a significant contribution to the country's energy needs. The United Kingdom agencies also participate in international energy research programmes, such as those of the European Communities and the International Energy Agency.

Just three days later Mr Benn's department launched a major research programme on wave power.

It was an unjustifiably inauspicious start to a period of great activity on the alternative energy front. Whether the MP was anticipating the announcement is not known. If he hoped to probe behind the veil shielding the department's Energy Technology Support Unit (ETSU), he did not have too long to wait before another attempt was made. Three top men from ETSU gave evidence the following week to the Energy Resources Sub-committee of the House of Commons Select Committee on Science and Technology. They offered little hint of the content of the Department of Energy's proposed research and development programme for existing and new sources of energy—that had to wait another week, until the Department of Energy's Chief Scientist, Dr Walter Marshall, appeared before the committee on behalf of the Advisory Council on Research and Development for Fuel and Power (ACORD). ACORD has now passed on that programme to Mr Benn.

But ETSU was not totally forthcoming. It confirmed that it was

placing contracts on behalf of the department and was thus playing a supervisory, monitoring role as well as performing its specified tasks on energy sources and energy conservation. ETSU also revealed its preferential ordering of alternative energy sources: wave power offered the "best potential contribution" and looked the most promising; solar came next, ahead of geothermal; wind and tidal power together took up the rear.

Without some sort of weighting, it all seemed rather crude. The subsequent ACORD hearing indicated that ETSU's chief concerns have been solar, geothermal and wind power; wave power studies were the province of the National Engineering Laboratory, while the Central Electricity Generating Board investigated tidal power. Obviously there is close collaboration, not least through the ubiquitous Dr Marshall and a plethora of committees. ACORD, in particular, is crucial. With members drawn from the nationalised fuel and power industries (coal, gas and electricity), from the oil industry, from research bodies (the SRC and the UKAEA) and from the academic world, it considers the results of ETSU's technical studies and advises Mr Benn on both research and development needs and the size of appropriate programmes drawn up by steering committees.

Thus it was able to confirm ETSU's preference to the select committee: it had considered tidal power (April–May 1975), and ordered a limited study of technical issues relating to the Severn Barrage; wave power (June 1975), which produced the £1 million feasibility study launched at the end of last month; geothermal energy (October 1975), any research programme on which needs to be integrable with the EEC's work in the area; wind energy (December 1975), about which a decision was deferred pending further study of costs; and solar energy (February 1976).

It was, in fact, solar energy's turn to capture some attention by this time, thanks to the publication of a massive document by the UK section of ISES, the International Solar Energy Society (see page 177). The bullish tone it adopts about solar energy prospects contrasts with the apparent view of ACORD. ACORD "accepts in principle the need for a national R & D programme" and wants a more detailed study, backed by research and development, "to define the options more precisely"—in other words, the case for solar energy, at

least as presented to it by ETSU, is not yet absolutely proven.

Whether all this reflects a genuine clash of views over priorities will probably only emerge after the one-day "national energy conference" organised by Mr Benn for June 22; ACORD's recommendations to Mr Benn are to be made public the same day. The 50 organisations invited to this more obvious example of open government include representatives of industry, the unions, consumer, conservation and environmental interests, and professional, academic and research bodies. In the meantime, the ISES document is an attempt to reinforce the case for solar energy. Clearly the circle of interests that the subject of energy now covers is widening fast.

So fast, in fact, that the issues involved in alternative energy seem to be growing increasingly political—partly thanks to Mr Benn, but principally because the potential of the various energy sources under scrutiny turns less on the well documented scientific evidence that can be brought to bear than on the impenetrable economic factors involved. The ISES document provides just one example: the case it puts for solar energy is based on more than mere scientific feasibility, but it is not really couched in the propagandist terms typical of a document urging policy changes. As one environmentalist privately pointed out, though, it does provide excellent ammunition.

Alternative energy, however, is but the tip of the iceberg, as any table of comparative expenditures on energy in Britain would quickly show. And as if to add to the recent excitement, the Energy Research Group at the Open University has churned out its own critique of the electricity industry, advocating a national fuel policy and suggesting that sheer overestimation of demand has produced a large measure of overcapacity. It can't be long before the fact that power is consumed to such a large extent in the form of electricity also becomes a legitimate area of enquiry.

Back in the committee rooms of the House of Commons, however, was the poor Science Research Council (SRC), also giving a view on alternative energy resources—the beleaguered Sir Sam Edwards was in the chair. He took the opportunity to cock a snook at the Treasury: in an obvious reference to the condition of "big" science under the SRC, he indicated that even if it was available extra money would not be devoted to alternative energy.

IN BRIEF

Explosions treaty delay

Work has been completed on the final draft of a US-USSR treaty limiting the size of so-called peaceful nuclear explosions, but President Ford has delayed signing the treaty to keep it from becoming an issue in some key Republican primary elections. Formal signing ceremonies, which were to have been held last week in Washington and Moscow, were dropped when Mr Ford suffered a string of defeats at the hands of Ronald Reagan, his right-wing challenger. Anxious not to give Reagan yet another foreign policy issue to snipe at, Ford decided to delay the signing until after the crucial May 18 Michigan primary. The irony in all this, however, is

that so far the treaty has been attacked almost solely by liberals who regard it as a sham in terms of arms control.

Nuclear trade moves

US pressure for the severest curbs on the sale of nuclear reprocessing and enrichment technology is expected when the "Group of Seven" (now swollen to ten) nuclear powers meets secretly in London next month. Reports indicate that US delegates will move to dissuade West Germany and France from going ahead with plans to export to Iran and Pakistan. They may also press for a cancellation of the West German-Brazilian deal agreed last year. Meanwhile, Congress is expected

to approve a request from the American General Electric Company to export two nuclear installations to South Africa.

New USSR group

A new dissident group, headed by physicist Yuri Orlov, has been formed in Moscow. The group aims to monitor the implementation in the Soviet Union of the Helsinki pledges on human rights. Dr Orlov was quickly arrested and informed that he and the other members of the group would be liable to legal action if they persisted with this "unconstitutional activity". Released after an hour, Dr Orlov stated that the group would continue to function.

Our authorities are right to be concerned about the risk of rabies being introduced into Britain. In recent years the disease has spread rapidly over Europe, and has now reached the coast of France just across the English Channel. All dogs entering the country have to spend 6 months in quarantine so those already infected may be detected; the main risk, however, is thought to be that a pet dog incubating rabies might be smuggled into England, either on a small boat which eluded the customs or, tranquillised, in the boot of a car. Our officials are increasingly alert to the danger, and heavier penalties are being given to those caught trying to evade the law, but the risk cannot be completely eliminated. It is also possible that rabies could reach Britain by other means. We do not have vampires, which are notorious carriers in Central America, but even the apparently harmless insectivorous bats, which are known to fly across the channel, are able to carry the virus. It would even be possible for a fox to swim the channel.

In France foxes have been the main carriers of rabies, and would probably be the greatest danger in Britain. There are therefore plans to kill all the foxes living in an area around any focus of rabies which might occur. Now that the fox is becoming a common suburban denizen in Britain, its potential as a disease carrier is increasing. So is the task of control if this becomes necessary. Foxes likely to endanger city dwellers live in overgrown cemeteries and disused railway embankments, and their elimination will be very difficult. On the other hand in rural areas, where foxhunting is popular, there should be less trouble. Contrary to the view put out by supporters of foxhunting, there would be little difficulty in

killing off every fox in their territories in a few days using gas, shooting and poison. Foxhunting is only a method of culling the surplus population, and mainly serves to conserve the species in many areas. The sport would have to be stopped if rabies appeared in the countryside, as the hounds and, in

Shooting the rabids**KENNETH MELLANBY**

turn, the hunters, would be at risk if they chased a rabid fox.

Wildlife conservation would suffer greatly if rabies became a serious problem in Britain. Not only foxes but many other animals, particularly carnivores like stoats and weasels, might be a danger, and there would be attempts to exterminate them. As there is already considerable pressure on these species in Britain, further persecution would probably be effective, and the whole balance between predators and their prey would be upset. The result might well be an unwelcome population explosion in undesirable species like the rat. The effect on the countryside might be

serious.

So far our preparations in Britain have been to stockpile vaccine and serum, to protect animals and to treat humans should rabies appear, and to make plans to try to wipe out foxes and other wild animals. Surely we should immediately devote our attention to our domestic pets? Although an infected fox might appear unnaturally tame, and infect a child (particularly one interested in wildlife) who played with it, a rabid dog would clearly be a much greater danger. While I would not wish to interfere with the right of the responsible pet lover to keep a dog, I think that we should take immediate action to eliminate the large numbers of uncared for, stray and feral dogs which are already a real danger to children and even adults in many parts of the country.

It is estimated that less than half the six million dogs in Britain are licenced, even though the fee remains ludicrously low at 37½ pence. These dogs will be more difficult to eliminate than foxes, so we should start at once. At present there is no requirement to licence a puppy under six months old. so many pets are bought for children only to be turned out when they become larger, hungrier and less attractive. Hundreds of thousands of animals are abandoned when their owners go on holiday. A vigorous campaign to destroy all unlicensed dogs, and a much more expensive licence, required even before a puppy could be acquired, would go far to solve the problem. Even without the risk of rabies a reduction in the number of unwanted dogs would make our cities cleaner, more hygienic and safer—and would cut down the need for expensive imports of high protein dog foods which might reduce hunger in other parts of the world.

correspondence

Class structure in science

SIR,—To be castigated in a leader column is for me a unique and not entirely pleasant experience, but one which, given the choice, I would rather have had for something I did say than for something I did not say.

In talking to the Research and Development Society on April 27 I was careful to say that I did not believe simple direction was the right way to harness the resources of university science to our national objectives. I made no suggestion that (to quote your leader) "research funding in universities should be approved only if the research has clear economic benefit". Nor did I say that "individual universities should be designated as centres of expertise for certain industrial research purposes".

I emphasized that to benefit more directly from university science was essentially a matter of changing the traditional attitudes towards research of other than the very pure kind, and said in this connection that it "would help, perhaps, as a conditioning mechanism, if all applications for funds for academic research were required to state what would come from its successful completion, with the words 'a better understanding of' or any paraphrase of those words banned". No question here of "clear economic benefit" or approval based on such a concept—just a suggestion that clear thinking about where a project fits into the scheme of things would help the attitude-changing process. My mention of banning the phrase "a better understanding of" was a half-humorous reference (well understood by the audience, in which research management was fairly strongly represented) to the fact that such a phrase is the resort of nearly all woolly proposal writers.

In suggesting that we might consider allocating a major interest area to each academic institution I was not thinking of industrial research and made no mention of it. The examples I gave of the kind of theme institutions might tackle were very broad divisions of our national life and the objects of the suggestion were

- to ensure that all aspects of our communal existence were being thought about
- to generate groups of people of different disciplines sharing a common interest in an important

national theme.

A journal like *Nature* which is looked upon as the epitome of scientific accuracy has a special duty to be certain of its facts. If there is a class war in science (which I doubt), your role, Sir, is that of *agent provocateur*.

I presented my talk not as a means of expounding specific proposals but to help in generating serious discussion on a vital topic. Your handling of it will almost certainly be prejudicial to that objective, but if any of your readers would like to know what I actually said I shall be happy to send them a copy. I will even send one to you, Sir, if you are interested in fact rather than fiction.

M. K. MCQUILLAN

Tewkesbury, UK

Frozen mineral waters

SIR,—In his article (March 18, page 182) Allan Piper makes it clear that as soon as the exploitation of the mineral wealth of Antarctica becomes a commercial proposition there will be a major political conflict. Surely the way to handle this situation is to tackle the problem now, while the commercial issues are still relatively remote. Yet I am very much afraid that the temptation will be to postpone any resolution of the situation until the problem arises, by which time it will, of course, be too late to avoid direct confrontation and very probably military intervention.

I believe that action is necessary now to defuse this situation; for example by an agreement to sign over all mineral rights on the continent to the United Nations Organisation. It is to be hoped that signatories to the Antarctic Treaty will not sidestep the issue at their meetings this year and next.

J. A. EADES

University of Bristol, UK

Allergic reactions

SIR,—Reports that animal handlers are allergic to laboratory strains of rats, mice and rabbits are becoming increasingly prevalent (Correspondence, March 25, page 280). In the pages of *Nature* and *Science*, the problem has been considered only from the human point of view; we bemoan the fact that many of our colleagues must either walk around with gauze masks or gulp antihistamines in order to survive their experimental subjects. We suggest that the time has come to view the in-

creased number of allergic reactions from the murine rather than human perspective. The rat is evolving; we are the catalysts speeding its evolution.

Consider an animal handler approaching a colony of rats, to some of whom he or she is allergic. Is it not natural for the handler to select for experimentation only those animals who do not cause an allergic reaction? Faced with a roomful of rats, who of us would willingly choose the rat which caused our eyes to tear, our nose to drip or our bronchi to constrict. We are selectively destroying non-allergenic animals leaving behind the allergenic to act as breeders. One can see additional problems arising in the future. Rats and mice are usually picked up by the tail. Just as selection for super-allergenic strains increases the rat's chances of survival, so does selection for the short-tailed or tailless rat.

If we must root around in the rats' cage for five minutes just to find his tail, we suspect that we'll either move to the next cage or suffer a bitten finger. In short, the super-allergenic, tailless rat is a problem only for the experimenter; from inside the cage it will undoubtedly be greeted with chuckles or cheers.

JACK A. KORNBLATT

MARY JUDITH KORNBLATT

Concordia University,
Montreal, Canada

Human anatomy

SIR,—Your correspondent Anthony B. Harris (May 6th, page 10) describes his "experiments" on young ladies purporting to determine a functional basis for structural asymmetry. His results, which suggest to him a positive correlation between handedness and asymmetry, are at complete variance with our observations on a thousand normal individuals, although asymmetry is certainly the rule rather than the exception.

Since Anthony Harris' measurements are all of circumference or volume, this may account for his inability to detect equivalent asymmetries in the two-dimensional representations of the human form to be found in the National Gallery; these asymmetries, however, are observable in sculpture of many centuries and cultures.

R. C. CONNOLLY

P. H. DANGERFIELD

University of Liverpool, UK

news and views

H-2 and HLA sequences

from J. C. Howard

ONE of the most important and illuminating observations in the convoluted history of the major histocompatibility complex (MHC) was the demonstration by Snell, Klein and Shreffler that the highly complex antigens of the H-2 system of the mouse could be largely resolved into two allelic series, one at each of two closely linked loci in the K and D regions of the chromosome 17. This observation emphasised the remarkable formal similarity between the principal leukocyte antigen series of man and mouse, and led to the hypothesis that the two allelic series represented products of related genes that had arisen as a result of ancestral duplication. This simplification in turn led to attention being concentrated on the products of these loci—the best defined genetically and serologically in the MHC—as appropriate subjects for biochemical characterisations. In the past few years progressively more detailed analyses of these antigens have led finally, in the past few months, to the first amino acid sequences, the most recent study of which appears in this issue of *Nature*¹.

There is now general agreement that both the H-2D and H-2K products of the mouse, and the HLA-A and HLA-

B products of the human MHC are cell surface glycoproteins of total molecular weight approximately 60,000 daltons. They carry about 3,000 daltons of carbohydrate on a heavy chain of approximately 45,000 daltons, and a non-covalently linked light chain of about 12,000 daltons which is known to be β_2 -microglobulin. The molecule is inserted into the membrane at the C-terminus, and the whole molecule is soluble only in detergent. A papain fragment of about 36,000 daltons carries all the antigenic activity (as well as the light chain), however, and is water soluble. It is likely that the native molecule exists in the membrane as a monomer, although a free sulphydryl group is available near the C-terminus and may normally participate in some dimerisation. A lymphocyte carries approximately 5×10^5 antigen molecules on its surface.

It has required considerable ingenuity to obtain amino acid sequences from H-2 and HLA antigens because of the minute quantities of material available. By using internal isotopic labelling with individual or small groups of amino acids, four American groups have obtained partial N-terminal sequences of three K end alleles and two D end alleles (Henning *et al.*²;

Vitetta *et al.*³; Silver and Hood⁴; Ewenstein *et al.*⁵). Dissociated immunoprecipitates from detergent-solubilised membrane preparations of normal lymphoid cells were sequenced in the Beckman automatic Sequenator, and the labelled residues identified with appropriate controls. Strominger and colleagues (Terhorst *et al.*⁶) and now Bridgen *et al.*¹ in this issue of *Nature* have obtained partial N-terminal sequences of HLA-A and HLA-B products from lymphoid cell lines. Bridgen and colleagues have adapted solid phase sequencing techniques of whole protein molecules, using a sensitive post-labelling method to identify consecutive residues autoradiographically on two-dimensional thin layer chromatography.

The sequences obtained by these various techniques are summarised in Fig. 1. They demonstrate beyond all dispute the common evolutionary origin of the D and K loci in H-2, and of the A and B loci in HLA. From the substantial numbers of shared residues between H-2 and HLA sequences, a common evolutionary origin for the genes determining both H-2 and HLA is also apparent. The existence of marked species specificity inside the H-2 and HLA sequences seems to

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Mouse H-2	K ^b	(Met)	Pro	His		Leu	Arg	Tyr	Phe	Val		Ala	Val		Arg	Pro		Leu	-		Arg	Tyr	Met					Tyr
	K ^k	(Met)	Pro	His		Leu	Arg	Tyr	(Phe)	His		Ala	Val		Ile	Pro		Leu	Lys	(Pro)	Phe	Ala	Met	His				Tyr
	K ^d	(Met)	-	His		-	Arg	Tyr	(Phe)																			
	D ^b		(Pro)	-		-	Arg	Tyr	-	-		Ala	Val		Arg	Pro		Leu	-		Pro	Arg	Tyr					
	D ^d	Met	(Pro)	His		Leu	Arg	Tyr	Phe	Val		Ala	Val			Pro		-	-		Pro		Tyr					
Human HLA	A2	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Phe	Thr	Ser	Val	Ser	Arg	Pro	Gly		Gly	Glu		Phe	Ile	Ala	Val			Tyr
	B7	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Tyr	Thr	Ser	Val	Ser	Arg	Pro	Gly		Gly	Glu		Phe	Ile		Val			Tyr
	A1,2								Phe	Phe	Thr	Ser		Ser														
	B8,13		Ser		Ser	Met	Arg	Tyr	Tyr	Tyr	Ser	Ala	Val	Ala	Arg	Pro	Gly											

Fig. 1 Collated H-2 and HLA sequences derived from the following sources: H-2, Henning *et al.*², Silver and Hood⁴, Vitetta *et al.*³, Silver and Hood⁷, Capra, personal communication; HLA, Terhorst *et al.*⁶, Bridgen *et al.*¹, Bridgen *et al.* sequenced a mixture of four HLA molecules giving rise to the alternative residue allocations in positions 8, 9, 10, 11 and 13. Residues in parentheses represent either preliminary or disputed results, dashes indicate that the residue present is not any of the other residues known at that position except in position 9 of H-2D where the residue is not valine but may still be histidine. Residues shared between different H-2 sequences are indicated by the vertical blocks, while residues shared between H-2 and HLA are similarly marked in the HLA sequences.

imply that the postulated duplication must have followed the evolutionary divergence of man and mouse, and yet the existence of paired serological loci in many species of mammal suggests the reverse. A resolution of this paradox may be that the genes specifying these antigens show a recurrent tendency to duplicate during evolution. This view might be supported by the existence of a third serological locus, HLA-C, in the human, although the homology of this locus with the A and B loci remains to be demonstrated. The allotypic differences between H-2 specificities at the D and K loci, and between HLA specificities at the A and B loci seem to be reflected in several sequence non-identities between different alleles, for instance at residues 2, 5 and 19 for the three H-2K sequences and residues 3, 5, 8, 9, 17 and 19 for the two H-2D sequences. The frequency of these allotypic substitutions is unprecedented in known allelic systems, and if it is sustained throughout the length of the molecules will pose considerable theoretical problems in accounting for their origin. The conventional models for origin of simple (that is, single substitution) allotypes impose intolerable genetic load on the species when multiple substitutions are to be obtained. Alternative mechanisms to account for the observed differences have been elegantly discussed by Silver and Hood in a recent review (*Contemporary Topics in Molec. Immunol.*, in the press)⁷.

It is curious that the sequences reveal no tendency for D products to resemble each other more than they do K sequences, or *vice versa*, since from serological studies the products of the two loci can be shown to differ by locus-specific public antigens. It seems likely however that further sequence data will ultimately confirm the serological results, since in all other respects the implications of serological data have been confirmed by the sequences.

For some time now the notion has been in the air that the remarkable polymorphism in histocompatibility antigens may reflect the specificity of some kind of recognition system, and hence the possibility that H-2 and HLA antigens may show sequence homology with immunoglobulin has been widely canvassed. The association of β_2 -microglobulin with these antigens as a "light chain" has reinforced this suggestion in view of the homology of this molecule with an immunoglobulin domain. In the event the sequences of H-2 and HLA antigens so far available do not support the immunoglobulin homology theory, and the absence of a half-cystine anywhere in the first 40 residues also argues strongly against homology. Nevertheless it is possible that sequence

homology with immunoglobulin will emerge later on, or alternatively, if the two genetic systems are very distantly related, sequence homology may be absent, but a three-dimensional homology sufficient to account for β_2 -microglobulin binding as a light chain homologue may be preserved (Hood, personal communication).

There is no doubt that the preliminary sequence data now available for H-2 and HLA antigens have raised as many intriguing problems about the organisation and evolution of these remarkable chromosome regions as they have resolved. It seems certain that as sophisticated microsequencing techniques are further developed there will continue to be a flood of new information about these and other molecules such as Ia or HLA-D in the MHC that will prove as fascinating and intellectually demanding in their interpretation as the immunoglobulin sequences have been.

¹ Bridgen, J., *et al.*, *Nature*, **261**, 200 (1976).

² Henning, R., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 118 (1976).

³ Vitetta, E. S., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 905 (1976).

⁴ Silver, J., and Hood, L., *Proc. natn. Acad. Sci. U.S.A.*, **73**, 599 (1976).

⁵ Ewenstein, B. M., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 915 (1976).

⁶ Terhorst, C., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 910 (1976).

⁷ Silver, J., and Hood, L., *Cont. Topics molec Immunol.* (in the press).

Ineffective nerve terminals

from Shin-Ho Chung

SEVERAL recent studies by Patrick Wall and his colleagues indicate that a class of synapses in the central nervous system is normally inactive but becomes effective if the target cells are deprived of their main inputs. If this notion is correct, it will have an enormous impact in neurobiology. The new concept will not only have a direct bearing on our understanding of how the brain adjusts to injury or altered environment, but may also shed new light on the handling of incoming sensory messages by the brain and the developmental strategies it adopts for forming intricate neuronal networks.

For many decades, anatomists and physiologists have explored the projection of sensory afferents onto the mammalian spinal cord. Together they have slowly compiled a story of almost incredible complexity. Cutaneous fibres originating from a circumscribed region of the skin (a dermatome) bundle together to form a dorsal root and enter the cord at the appropriate spinal segment. Once these fibres enter the cord, they branch out explosively to synapse with thousands of neurones in the grey matter. A fibre initially

divides into a T-shaped junction, and the two main stems, traversing several segments in the rostral and caudal directions, drop their numerous collaterals onto the cell layers beneath. It has been estimated that major collaterals are given off from the stems at intervals of 100 to 200 μ m. Since the radiation of sensory fibres continues over a region covering many segments, several hundred major collaterals can stem from any one fibre.

In apparent contradiction to these complicated, diffuse patterns of interconnections between sensory afferents and spinal neurones, physiologists have revealed a strict somatotopic map of the skin in the spinal cord. Each dermatome is represented by one or at most two segments of the spinal cord. Cells located medially in the dorsal horn synapse with fibres representing the distal part of the dermatome, whereas those located more laterally represent the proximal part of it. For example, each large cell in lamina IV (to which the large myelinated fibres carrying cutaneous information project) subserves a small patch of the skin, usually less than the size of one toe, and gentle pressure or movement of hair within this receptive field causes a vigorous discharge on that cell (Wall, *J. Neurophysiol.*, **23**, 197; 1960; Brown and Fuchs, *J. Neurophysiol.*, **38**, 1; 1975). Stimuli applied to areas outside this skin region fail to show any effect on the cell, despite the anatomical evidence for a widespread dissemination of sensory information within the cord.

This discrepancy between anatomical and physiological descriptions of the spinal cord has been a persistent worry to Wall. For if a single fibre spreads its terminals over 10 spinal segments, it is not immediately apparent how cells in one segment can have a restricted dermatome and maintain a strict somatotopic order within themselves. Wall and Werman (*J. Physiol., Lond.*, **255**, 321; 1976) have recently re-examined the central projection of spinal afferents and have confirmed the earlier anatomical descriptions. Using the technique of antidromic stimulation, they have traced the trajectory of single axons in the spinal cord of the cat. Some myelinated fibres invading the cord at Lumbar 2 segment, for example, have their terminals extended as far caudally as Sacral 1, which is 6 segments away from their zone of entry. Stated conversely, cells in a given segment receive not only fibres from their nearest dorsal root but also distant arborisations radiating from many neighbouring dorsal root fibres.

According to Wall (*Phil. Trans. R. Soc. Lond.*, in the press), the synapses formed by distant branches normally remain dormant, but become

active as a result of changes triggered by de-afferentation. This assertion is based on several related observations on lamina IV cells after cutting the appropriate dorsal roots. All fibres which form effective synapses with the cells are contained in the dorsal root nearest to them; if this root is cut, the cells become unresponsive to tactile stimulation, and the dermatome which is subserved by it becomes insensate. Merrill and Wall (*J. Physiol., Lond.*, **226**, 825; 1972) first discovered that within a few hours after surgery, de-afferented cells begin to respond to electrical stimulation delivered to neighbouring dorsal roots, although natural stimulation anywhere on the skin cannot excite them. One week after de-afferentation, the cells can be excited by light brushing, touch or pressure on regions of the skin which they normally would not represent (Basbaum and Wall, *Brain Res.*, in the press). Some of these cells are connected to two disparate parts of the body, for example, abdomen and leg. Hints of a similar phenomenon exist elsewhere in the mammalian brain. Cells in the thalamic nucleus of the rat (Wall and Egger, *Nature*, **232**, 542; 1971) and in the gracile nucleus of the cat (Millar *et al.*, *Expl Neurol.*, **50**, 658; 1976) change their receptive fields from leg to arm or trunk when the pathways connecting the leg to these nuclei are destroyed. These findings, taken together, do indeed suggest that axons in the brain form many more synaptic contacts than are apparently needed in normal circumstances. It should be noted, however, that various other interpretations, of which sprouting of neuronal terminals into vacated territory is one, have not been conclusively eliminated.

There are several possible ways in which a synaptic terminal could be rendered ineffective. Nerve impulses travelling down an axon may invade only a few proximal branches and fail to invade the remaining terminals of the axon. Alternatively, the distant terminals may terminate at distant segments of the dendrites of their target cells, thereby making the contacts less effective in bringing the target cells to the threshold of firing. Finally, the distant terminals may terminate on their target cells not directly but through small interneurons. In any of these cases, changed conditions in the tissue induced by de-afferentation could conceivably cause the presence of such distant terminals to be more apparent.

Whatever the mechanism ultimately turns out to be, Wall's findings pose the intriguing possibility that during development a growing axon forms diffuse contacts with a large number of target cells, and through use and disuse some of these contacts remain

effective whereas the others become ineffective. The ineffective terminals would remain, in Merrill and Wall's phrase, as "ghosts of the cell's childhood" and become functional again if the neuronal circuitry needed to be modified. This line of speculation leads to testable predictions about early developmental processes of somatotopic organisation. For example, a lamina IV cell in a young kitten could be excited by sensory fibres stemming from disparate regions of the skin, and ordered representation of cutaneous receptive fields onto spinal neurones could evolve gradually as the animal matures. Now and for the next few years, as more direct approaches are used to reveal the mechanisms governing development and plasticity of the nervous system, the observations of Wall, and the interpretations he has drawn from them, will remain as a valuable guide. □

Brightness variations of Saturn's Rings

from David W. Hughes

ASTRONOMERS, since 1921, have occasionally reported that the brightness of Saturn's rings varies with azimuthal angle (the angle θ in Fig. 1). The degree of variation is a matter of considerable debate, however, and papers have been published claiming that the eastern ansa of the inner ring B is brighter than the western one, fainter than the western one, and that each ansa is fainter than the ring position directly in front of Saturn (that is $\theta=90^\circ$). The outer ring A is also found to vary in brightness, but in a more complicated way.

Hoping to solve this ambiguity Karl Lumme and William Irvine of the Department of Physics and Astronomy, University of Massachusetts, have made careful photometric studies of some 100 good-quality Saturn plates from the New Mexico State University and Lowell Observatory collections. The preliminary results are reported in a recent edition of the *Astrophysical Journal* (**204**, L55; 1976). The brightness of each ring as a function of θ has been examined for each of six wavelengths in the band 3,600 to 8,000 Å and also for phase angles, α , (the angle Earth-Saturn-Sun) larger or smaller than 1° and for ring tilt angles, B , larger or smaller than 20° . Results show that at 5,500 Å, $\alpha > 1^\circ$ and $B > 20^\circ$, the outer ring A has a maximum of brightness at $\theta=45^\circ$ and 225° and a minimum (about 10% lower) at $\theta=135^\circ$ and 315° . The effects seem to be less pronounced at shorter wavelengths.

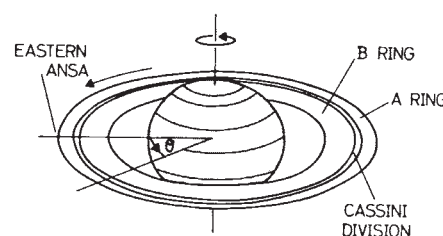


Fig. 1 Saturn and its rings. The brightness of the A ring varies with θ , the azimuthal angle, whereas the B ring seems to have a constant brightness.

For the inner B ring Lumme and Irvine find small maxima in brightness for $\theta=0^\circ$ and 180° . This is caused by the image spreading on the photographic plate due to the finite resolving power of the telescope, atmospheric effects and photographic irradiation on the plate. When corrections are made for this spreading, the resulting brightness is found to be independent of θ .

The authors conclude from their results that the particles in ring A may have rotation periods locked to their period of revolution about Saturn. So just like the Moon they always point the same face to the central planet. The above data also indicate for the first time that the average properties of the particles in ring A may differ from those in ring B.

Morrison (*Icarus*, **22**, 57; 1974), who studied the rings by infrared radiometry, found that the particles at the western ansa had a higher brightness temperature than those at the eastern ansa. He proposed that the particles have uneven albedoes, the brighter sides being orientated towards their direction of orbital motion.

The nature and size of the particles in the rings are still poorly understood. Pollack (*Space Sci. Rev.* **18**, 3; 1975) reviewed recent visual, infrared, radio and radar data and concluded tentatively that the mean particle size is around 10 cm, the bulk composition being, in the main, water ice. These particles occupy between 0.5 and 1% of the total volume of the rings. The physical thickness of the rings is about 100 m but the rings do bulge slightly at some of the period resonances with satellites Mimas and Titan. The total mass of material in the rings is small, Pollack's figures leading to a value in the region of 10^{19} g, which is about the mass of a large comet and is over a million times less massive than the Moon.

It is hoped that this picture will be greatly clarified in the next decade when the data from the Pioneer and Mariner Jupiter/Saturn space probes have been received and analysed. □

DARWIN developed his theory of sexual selection to account for the extravagant secondary sex characters possessed by males of many species. Peacock tails, deer antlers, and bird of paradise plumes are classic examples of characters which hamper survival, but which may increase mating success. Darwin's theory was neatly formalised by R. A. Fisher 36 years ago in his book *The Genetical Theory of Natural Selection* and later developed as a genetic model by O'Donald (*Heredity*, **17**, 541; 1962); briefly, the argument goes like this. Females on the whole tend to be fussier in their choice of mates than are males (the female has more to lose if she makes a bad choice because eggs are more expensive than sperm), so that males end up vying with one another for the attention of a female. Females will always try to choose partners of 'good quality'—those which make good parents, or which have good genes to pass on to their children—and in the course of evolution females will develop a preference for external indicators of these qualities. Now suppose that females have begun to evolve a liking for males with green heads, an indicator of good quality: immediately a new selective force comes into play. Males with green heads will now be at an advantage because they are popular with females, and therefore females which choose green-headed males will have sexually attractive sons (assuming inheritance of the green colour), regardless of the quality of these sons in any other respect. The female preference itself generates a selective advantage for green heads, which in turn favours a stronger female preference. It is this positive feedback loop which has

Sexual selection and the handicap principle

from John Krebs

led to the extraordinary elaboration of many male sex characters.

Recently, Zahavi (*J. theor. Biol.*, **53**, 205–214; 1975) has come up with an audacious alternative theory of sexual selection. He argues that the females' taste for males with costly, survival-reducing adornments evolves precisely because these features are a handicap. A male which has survived to breeding age in spite of dragging around a huge tail since birth has overtly demonstrated his superior fitness, just as if two athletes ran a mile in four minutes, but one carried a sack of coal, we would conclude that this individual was stronger than his rival. The handicap principle, as Zahavi calls it, has an immediate intuitive appeal. A female benefits by preferring the most handicapped male, because he will pass on good genes to her children, and the handicapped male benefits because the heavy cost of his adornments prevents easy copying by other males. Unfortunately, however, the handicap principle does not work. Maynard Smith (*J. theor. Biol.*, **57**, 345–354; 1976) and Davis and O'Donald (*J. theor. Biol.*, **57**, 239–242; 1976) have both analysed Zahavi's idea in more detail, and they show (as many people had suspected) that the verbal argument has serious flaws when translated into strict genetic terms.

Both critiques point to an obvious

problem. Even if a handicapped male passes on good genes to his children, he unfortunately also passes on his handicap, which must, according to Zahavi's hypothesis, reduce their survival chances. Perhaps the idea can be salvaged by assuming that the good genes are passed to male and female offspring, but the handicap only goes to the male? Davis and O'Donald point to two difficulties with this argument. First, it is unlikely that genes which are good for a highly adorned male will also be optimal for a cryptic female, and second even if one assumed that the same genes were optimal for both sexes, selection would soon bring females to near their optimum genotype, at which point the advantage of choosing a handicapped male would fade away.

Maynard Smith puts Zahavi's verbal argument into the form of a simulation model, using three loci, one for fitness (high and low), one for a handicap such as tail length, and one for female choosiness—the choosy allele only mates with handicapped males. The fitness of a 'high fitness—no handicap' male is set at 1, and the other fitnesses are calculated by subtracting a cost of the long tail, and a cost of the 'low fitness' allele. In order to eliminate the effect of female preference (Fisher's idea) the simulation was started with a very low frequency of the 'choosy' allele, the other loci being represented by equal frequencies of their two alleles. The results of the simulation were unambiguous: choosy females and handicapped males declined in frequency, and this was true for a wide range of fitness values. The handicap itself does not favour the evolution of elaborate male sex displays.

Interferon deglycosylated

from David Metz

INTERFERON is the cellular protein, synthesised by virus-infected animal cells, which effects the protection of other as yet uninfected cells against subsequent virus infection. Interferon has long been believed to be a glycoprotein. The carbohydrate substitution is to be expected of a secreted protein though the experimental evidence for its occurrence has hitherto been somewhat indirect. The principal impediment to the full biochemical characterisation of the various molecular species of interferon has been the difficulty of purifying the material to demonstrable homogeneity. The reason for this difficulty lies in the very high specific activity of interferon which means that

large volumes of crude preparations (necessarily from tissue culture) yield tiny amounts of pure interferon.

Progress in purifying interferon has been steady if unspectacular in recent years. The current state of the art is well illustrated by two recent papers from Knight on the purification and characterisation of mouse and human interferons. Using basically similar purification procedures, both mouse L cell interferon and human diploid fibroblast interferon were purified to specific activities of 2×10^8 units per mg protein. The human interferon preparation, when characterised by polyacrylamide gel electrophoresis, displayed a single peak containing essentially all the interferon activity, protein staining capacity and carbohydrate staining capacity of the preparation (Knight, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 520; 1976). This preparation, containing a

single polypeptide of apparent molecular weight 20,000, would seem to be the first homogeneous preparation of human interferon.

In contrast, the mouse interferon yielded some dozen discrete bands of activity, following polyacrylamide gel electrophoresis, spanning an apparent molecular weight range of 20,000 to 32,000. About half of these components were demonstrated to contain carbohydrates, as judged by the periodic acid-Schiff base staining procedure (Knight, *J. biol. Chem.*, **250**, 4139; 1975). The reason for this heterogeneity of mouse interferon is as yet undetermined. Variation in the carbohydrate content, as opposed to the primary amino acid sequence, is clearly one possibility.

A second type of heterogeneity in which a variable degree of glycosylation appears to be implicated is the

charge heterogeneity of interferon which may be examined by isoelectric focusing. Dorner *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1981; 1973), confirming the earlier work of Schonke, Billiau and De Somer, demonstrated that treatment of rabbit interferon with neuraminidase converted the native heterodisperse charge distribution to a single component with no loss of biological activity. Since neuraminidase would be expected to cleave a terminal sialic acid residue from a penultimate galactose in a carbohydrate side chain it was inferred that native rabbit interferon contained variable amounts of sialic acid as the terminal sugar which, moreover, was not involved in the antiviral function of the molecule.

This type of investigation has recently been extended to human interferon by Anfinsen and his colleagues (Bose *et al.*, *J. biol. Chem.*, **251**, 1659; 1976) who have shown that 80% of the sugar moieties may be removed from a partially purified interferon preparation using a mixture of glycosidases, again without any loss of biological activity. Unfortunately, since the interferon preparation used was of undefined purity one cannot estimate how much of the carbohydrate of the interferon molecule itself was removed in this experiment. Nevertheless, the demonstration of both size and charge changes of the interferon activity following enzymic digestion do support the notion that at least some of the sugar residues may be removed from human interferon without loss of biological activity.

If it turns out that all the carbohydrate is dispensable without loss of activity the eventual chemical synthesis of interferon would be facilitated. But whether such a synthetic molecule free of carbohydrate side chains would be therapeutically useful is uncertain. One general feature of studies in which interferon has been used to attempt the protection of man and animals against virus challenge has been the relatively large quantities of interferon required for efficacy. This may well be a consequence of the common finding that interferon has a short half-life in the circulation. It is known that a variety of glycoproteins are rapidly cleared from the circulation when even a limited number of the normal terminal sialic acid residues are removed (Ashwell and Morell, *Advances in Enzymology*, **41**, 99; 1974). Thus Dorner *et al.*, (*op. cit.*) have suggested that the half-life, and consequently the therapeutic usefulness, of interferon might be enhanced if the native heterogeneity were diminished by the additional incorporation of sialic acid residues. So reglycosylation of synthetic or natural interferons might in fact enhance their therapeutic value. □

Cross section for nuclear fusion

from P. E. Hodgson

WHEN two nuclei collide, there is a certain probability, depending on the energy and on the nuclei, that they will fuse to form a compound nucleus, which subsequently decays by the emission of neutrons, charged particles and gamma rays. In recent years there have been many studies of the cross section for nuclear fusion, and some features of the process are now understood (*Nature*, **256**, 261; 1975).

The probability that two nuclei fuse depends on the impact parameter, the shortest distance between their initial directions of motion. If the collision is a glancing one (large impact parameter), a few nucleons may be transferred from one nucleus to the other, or knocked out entirely, but the nuclei will go essentially unchanged. If the collision is more or less head-on (small impact parameter), the nuclei will interact strongly and are likely to fuse.

The probability of fusion also depends on the energy of the collision: at low energies the nuclei are kept apart by the electrostatic repulsion and no reactions can occur. As the energy increases, so does the fusion probability, until at very high energies it begins to decrease because the nuclei are more likely to be shattered by the violence of the impact.

These collisions are far too complicated to be treated in detail by quantum mechanics, but substantial progress has been made towards understanding the fusion cross section by using classical ideas (*Nature*, **256**, 261; 1975). If R is the impact parameter, then the angular momentum of the lighter ion about the heavier is

$$L\hbar = mvR = k\hbar R$$

If all collisions with impact parameters less than R lead to fusion, then the total fusion cross section

$$\sigma_f = \pi R^2 = \pi^2 L^2 / k^2$$

As the energy increases, the peripheral collisions bring in more angular momentum than the compound nucleus can accept, so fusion can only occur for angular momenta less than some limiting value $L_F < L$ and then

$$\sigma_F = \pi^2 L_F^2 / k^2$$

Very many experimental studies of the fusion of nuclei have now been made, and it is found that the fusion cross sections behave in a systematic way. In particular, measurements have been made of the fusion cross section for several different pairs of nuclei giving the same compound nucleus at the same energy, and also of the same reaction at a series of energies. These

have shown that fusion is not a static phenomenon; it depends on the dynamics of the interaction process.

Conditions required

A careful analysis of a wide range of fusion cross sections by Galin and colleagues (*Phys. Rev.*, **C9**, 1018; 1974) led to the remarkably simple conclusion that all the data could be accounted for by assuming that fusion takes place as soon as the interacting nuclei come closer than a distance $1.1 (A_1^{1/3} + A_2^{1/3})$ fm where A_1 and A_2 are the atomic weights of the two ions. A similar analysis of data on other nuclei was made by Gutbrod and colleagues (*Nucl. Phys.*, **A213**, 267; 1973) and they came to a similar conclusion, but with the difference that the distance that fitted their data was $1.4 (A_1^{1/3} + A_2^{1/3})$ fm. These two results are significantly different and it is important that they be reconciled.

A theory that does this has been developed by Glas and Mosel (*Nucl. Phys.*, **237**, 429; 1975). It provides a simple model of the fusion process by assuming that the potential in the region of interest has a parabolic form with depth $V_B + \hbar^2 L(L+1)/2\mathcal{I}_B$ where L is the orbital angular momentum and $\mathcal{I}_B$ the nuclear moment of inertia. This enables the fusion cross section to be calculated analytically, and the formula obtained fits all the experimental values. At low and high energies, it gives to a good approximation the values found by Gutbrod *et al.* and Galin *et al.*, and thus unifies them coherently.

The reason for the change of slope is connected with the upper limit to the relative angular momentum of the two nuclei for which fusion can occur. This upper limit is set in two ways, and since both limits must be obeyed it is the lower of the two that actually

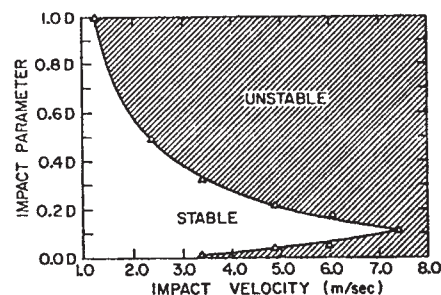


Fig. 1 Diagram showing the ranges of impact parameters and impact velocities leading to the fusion of two equal drops of water (unshaded). The impact parameters are expressed as fractions of the drop diameter $D = 300 \mu\text{m}$. The power limit to fusion at 3.4 m s^{-1} has a counterpart in nuclear collisions.

determines the fusion cross section. The first limit is set by the nuclear radius R ; the angular momentum cannot exceed $m\nu R$, where m and ν are the mass and velocity of the lighter of the two ions. In practice glancing collisions do not lead to fusion so the limit is somewhat less than this and allowance must also be made for the reduction of the velocity of the incident nucleus by the Coulomb repulsion.

The other limit is set by the maximum angular momentum that can be given to the nucleus without causing it to fly apart. This is fixed by the internal structure of the nucleus and can be estimated by the liquid drop model. At low energies this limit is not important, and the fusion cross section is limited by the nuclear radius. As the energy increases, the angular momentum $m\nu R$ increases until it reaches the critical value set by the limit of nuclear stability, and thereafter the fusion cross section is limited by the latter criterion.

These two limits correspond to the two different formulae for the separation between the nuclei for fusion to take place, and the transition region to the value of the angular momentum for which the two limits are approximately the same.

The theory of Glas and Mosel thus very neatly accounts for the variation with energy and with interacting nuclei of all the fusion cross sections available when they completed their work. Since then, however, further results have become available that cannot be accounted for by their theory, at least in its original form. The reason seems to be quite well understood in one case, but not in the other.

The first set of anomalous results was obtained by Gauvin and collaborators (*Phys. Rev.*, **C10**, 722; 1974). In the collision of two quite heavy nuclei, ^{76}Ge and ^{84}Kr , fusion takes place at a higher energy than is expected from the results for the collision of ^{40}Ar with ^{136}Sn , which leads to the same compound nucleus. This energy shift is as high as 15 MeV and was totally unexpected and quite outside the uncertainties of the experiment.

A possible explanation of this anomaly is that there is a lower as well as an upper limit to the impact parameter for fusion. It is then easy to adjust the lower limit to give the observed threshold shift, but then one wants to understand why fusion cannot take place for low impact parameters and how this varies with energy and from one nucleus to another.

Liquid drop model

A way of doing this has recently been suggested by Natowitz and Namboodiri (*Phys. Rev.*, **C12**, 1678; 1975), making use of the analogy with the collision of two liquid drops. This has

been studied by Adam and colleagues (*J. appl. Phys.*, **39**, 5173; 1968), and they did indeed find that at some energies there is a lower as well as an upper limit to the impact parameter for the fusion of the two drops.

Some of their results are shown in Fig. 1, which gives the impact parameters (expressed as fractions of the drop diameter D) for fusion as a function of the impact velocity in m s^{-1} . This diagram is easily understood: fusion takes place for low velocities and impact parameters but if either or both are large the drops either brush past each other or shatter.

The most interesting region occurs for low impact parameters and impact velocities greater than 3.4 m s^{-1} : even though the impact parameter is small fusion cannot take place, and this is just the effect that we want to explain in the collisions of heavy nuclei.

This effect can be understood by considering the collision in more detail. When fusion takes place, the kinetic energy of the colliding drops is transformed into the rotational and vibrational energy of the combined system. Now the kinetic energy can only go into rotational energy if the impact parameter is greater than zero, and the higher the impact parameter the more rotational energy can be given to the compound system.

For the extreme case of a head-on collision (zero impact parameter) the incident energy can be entirely transformed into vibrational energy for low impact velocities, but at the critical velocity of 3.4 m s^{-1} this is no longer possible. This corresponds to the maximum energy that can go into vibrations of the compound system without breaking it up.

If now the impact parameter is increased, some of the energy can go into rotational energy, so fusion can again take place. Thus for impact velocities above the critical value there is a lower limit to the impact parameter for fusion; below this impact parameter the system cannot be given enough rotational energy to absorb the energy remaining from the incident kinetic energy after as much as possible has been put into vibrations. At still higher impact parameters the rotational energy is so large that the two nuclei cannot stick together and fusion again cannot take place. There is thus a limited range of impact parameters for which fusion can occur.

As the energy increases, so does the rotational energy for a particular impact parameter, so that the lower limit to the impact parameter for fusion rises and the upper limit falls. Eventually at 7.4 m s^{-1} they coincide, and for higher velocities fusion cannot take place at all.

The lower limit to the impact para-

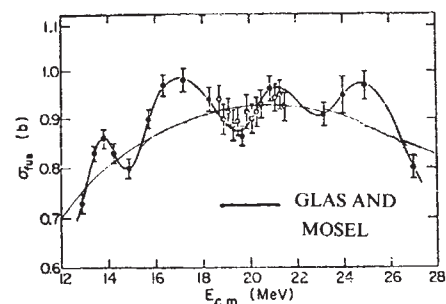


Fig. 2 Fusion cross section for the interaction of ^{12}C and ^{16}O showing the unexplained oscillatory structure superposed on the dependence given by the theory of Glas and Mosel.

meter for nuclear fusion can thus easily be understood by considering the behaviour of liquid drops. One important difference is the charge on the nuclei which prevents fusion at smaller impact velocities. For light nuclei the fusion threshold is below the critical impact velocity and so all impact parameters are allowed. The earlier experiments were all in this region and it was here that the systematic behaviour already mentioned was established.

For heavier nuclei, however, the fusion threshold is above the critical impact velocity and so only a limited range of impact parameters leads to fusion. There is thus an energy shift in the curve of fusion cross section as a function of energy compared with the curve that would have been obtained if the lower impact parameters also led to fusion. This higher fusion threshold is just what is observed experimentally for collisions between heavy nuclei.

The second set of data that cannot be understood by the theory of Glas and Mosel is the fusion cross sections of the interaction $^{12}\text{C} + ^{16}\text{O}$ from 13 to 27 MeV measured by Sperr and colleagues (*Phys. Rev. Lett.*, **36**, 405; 1976). As shown in Fig. 2, the experimental fusion cross sections oscillate around the mean value given by the theory of Glas and Mosel, and it is not at all clear how this behaviour can be explained. One possible explanation, that it is due to the effect of successive partial waves as they come in turn to dominate the reaction, is ruled out because it gives oscillations that have too small a period and too small an amplitude.

Other explanations are that it is due to the effect of resonances in other channels, or to a resonant transfer process (*Nature*, **246**, 14; 1973). According to this explanation, a nucleon can be transferred back and forth between the two approaching nuclei, and this could produce a periodic modulation of the fusion cross sections. This mechanism gives a very characteristic variation of the fusion cross section with energy, and this is approximately in accord with the experimental data. □

review article

Short term screening tests for carcinogens

Bryn A. Bridges*

There are now short term tests with a high predictive value for mammalian carcinogens. Many of them are based on the ability to detect damage to DNA in bacteria or mammalian cells after metabolic activation by microsomal enzymes. Their introduction will enable provisional safety assessments to be made for the many thousands of industrial and environmental chemicals for which long-term animal testing cannot at present be considered.

It has been estimated¹ that if one could totally abolish human cancer it would add a mere two years to the average lifespan. Most cancer sufferers are past retiring age so that industrial production would be little affected by the abolition of cancer. The fight against cancer must instead be justified in terms of the cost of hospital services and of basic humanity; treatment of cancer, even when it is successful, is a miserable process. When it fails, as it so often does, one feels guilty of a double offence, not only the loss of the patient, but the imposition of heroic measures that themselves may cause considerable physical and mental suffering.

The International Agency for Research on Cancer holds it as a rule of thumb that around 80% of cancer has an environmental cause^{2,3}; others would give a higher figure⁴. The evidence is indirect, being based on differences in tumour incidence between genetically similar populations in different environments^{2,3,5}. Even if this estimate is only approximately correct it leads ineluctably to the conclusion that a substantial proportion of cancers, possibly a majority, are in principle preventable. In past decades those responsible for the disbursement of cancer research funds have tended either to look for a breakthrough in the area of curative treatments or to make a long term investment in basic biology in an attempt to understand the disease (or more properly diseases since "cancer" is but a general term for hundreds of different malignant conditions). Recently, however, these two essential approaches have been complemented by a third, the search for the specific environmental factors involved in carcinogenesis.

The nature of these environmental factors is not known in detail, but it seems likely that many of them are man-made or natural chemicals. Even factors such as diet or stress may act indirectly by altering the metabolism of chemicals in the gut or in the body itself. Of course, identification of environmental carcinogens does not necessarily lead to their removal but it does open the way to control so that the risk that they present is no more than is necessary when weighed against any benefits that they may give.

The most direct method of identifying environmental carcinogens for man is based on population studies, but unfortunately it is expensive and seems to have rather low resolving power. Only a handful of chemicals are known to be carcinogenic to man and most of these have been detected following the study of workers occupationally

exposed to chemicals capable of giving rise to specific and rather rare neoplasms. The classic case is soot which has been known for 200 years to produce scrotal cancer in young chimney sweeps⁶. More recent examples are 2-naphthylamine, vinyl chloride and asbestos which produce, respectively, rare cancers of the bladder, angiosarcomas of the liver, and mesotheliomas of the lung cavity. The problems involved in identifying two populations differing only in their exposure to one chemical are formidable and are further compounded if the chemical gives rise not to specific and otherwise rare tumours, but to a variety of common cancers. Population studies are thus likely to be of limited value in identifying environmental (as distinct from occupational) carcinogens but they will be indispensable in providing the basis for risk evaluation, particularly where dose-response data can be obtained.

The alternative is to screen chemicals to which man is exposed. The generally accepted method of doing this is to carry out long term carcinogenicity tests with laboratory mammals. Not only are those tests very demanding of resources but any extension of animal testing on such a wide scale would be vigorously opposed by a number of animal welfare lobbies. In practice, it is inconceivable that resources could be made available (either men, money or mice) on the necessary scale to screen all the tens of thousands of substances to which humans are exposed. Of necessity, therefore, testing with whole mammals will be restricted to certain groups of suspect substances, for example those suspect but already in use on a large scale, or those substances which it is proposed to administer on a large scale, as food additives or cosmetics, for instance.

If one is to screen for carcinogenic chemicals, therefore, one must use short term tests with a high predictive value. I propose to review a number of possible systems which have been suggested in recent years. As will become apparent, many of them are in fact systems for the detection of agents causing damage to DNA. Damage to DNA leading to heritable changes may be important to man not only because of carcinogenicity but because it may cause hereditary disease⁷⁻⁹. Moreover, DNA damage may conceivably be involved in ageing and diseases associated with ageing¹. I take it as self-evident that any agent likely to damage the DNA of man, whether in somatic or germ cells, is potentially hazardous.

Screening systems

The induction of cancer is but one aspect of long term toxicity and for the evaluation of such hazards a three-tier

* Address: MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, Sussex, UK

approach has been proposed^{10,11}. The first tier would consist of simple short term sub-mammalian tests with a high predictive value for the human effect ultimately of interest. As many substances as possible should be screened with these tests. Second-tier tests would be both short and long term, on mammals. Only selected, high priority substances would be screened by these tests in addition to the first-tier tests. Tests in the third tier are designed not to detect toxic agents but to evaluate as quantitatively as possible the hazards to man from agents shown to be potentially toxic. Only substances whose use or presence seems inescapable would be subject to the third tier of evaluation, the object of which would be to make a risk-benefit assessment and institute appropriate regulatory action. Tests in successive tiers show in principle increasing relevance to man but this is often accompanied by decreasing sensitivity and practicability.

Not all of the sub-mammalian tests depend upon the postulated electrophilic nature of the active forms of carcinogens and in particular on their ability to react with DNA. Williams and Rabin¹², for example, have proposed that substances might be screened using a test based on membrane-polysome association. They found that a number of carcinogens caused degranulation of rough endoplasmic reticulum (microsomal membranes) from male rat liver. This test has been further developed by Purchase and Lefevre¹³ who have measured the loss of radioactive RNA from rough endoplasmic reticulum. Preliminary results (D. Anderson *et al.*, unpublished) with a large number of carcinogens and non-carcinogens indicate that the method predicts the activity of arylamines rather well (85% correct) although it is less successful with polycyclic hydrocarbons and direct acting alkylating agents.

The necessity for metabolic activation of many carcinogens by microsomal enzymes prompted the suggestion of McPherson *et al.*¹⁴ that the specific *in vitro* enhancement of biphenyl 2-hydroxylation activity in rat liver microsome preparation might be used as a screening test. They found that of eight known carcinogens, all caused an increase of around 100% in such activity, four compounds whose carcinogenicity is in doubt gave lower but significant increases, and eleven non-carcinogenic compounds gave no significant increase. This test system, like that of degranulation of ribosomes, is obviously promising and in need of a much more exhaustive validation on a scale similar to that used with some other systems.

Metabolic activation

There is a widespread belief among cancer workers that DNA damage is involved in the induction of cancer. That is the basis for the supposition that carcinogens might be detected by the consequences of DNA damage in simple systems. Two recent developments have enabled this possibility to be realised. First, it has become clear that many carcinogens are the products of metabolism of inactive chemicals by mixed function oxidases in the animal¹⁵, and that preparations of liver microsomes can be used *in vitro* to carry out this metabolic activation¹⁶⁻¹⁸. Second, ultra-sensitive bacterial systems, usually involving strains deficient in DNA repair, have been developed for the detection and characterisation of agents causing damage to DNA¹⁹⁻²³.

The first published work in which the mutagenic activity of metabolites was detected after metabolic activation of carcinogens was by Malling¹⁷. The methodology of his quantitative liquid assay system has been recently described²⁴. Later, Ames *et al.*²⁵ showed that microsomes could be added to the semi-solid agar overlay in a plate test, a procedure that is in some ways rather better for routine screening although it fails with a few compounds, for example, dimethylnitrosamine, possibly because the agar interferes with the diffusion of short-lived active metabolites.

Bacteria deficient in repair of DNA are killed more

easily by DNA-damaging agents than are wild type bacteria, and this is the basis for several simple tests. Bacteria deficient in excision repair have been used¹⁸ but these are sensitive only to certain types of DNA damage. Much more useful have been bacteria lacking DNA polymerase I (Pol⁻)²⁶, or deficient in genetic recombination (Rec⁻)²⁷. These tests are usually conducted on the surface of agar plates but are also amenable to rather more quantitative procedures with liquid-phase treatment^{28,29}.

Another way of revealing the existence of DNA damage is to look for the repair that it usually initiates and this is the basis of a very useful test developed by Stich and his colleagues. It depends on estimating the amount of DNA synthesis involved in repair by measuring autoradiographically the uptake of tritiated thymine during the period immediately following exposure to the test chemical. The method has the advantage that it can be used with cultured human skin fibroblasts. To prevent normal DNA synthesis the cells are kept in an arginine deficient medium for 3 d before exposure.

In a report on 64 substances tested, Han and Stich³⁰ found that all directly acting carcinogens elicited unscheduled DNA synthesis whereas no repair synthesis was observed after treatment with 16 non-carcinogens. Most carcinogens known to need metabolic activation gave negative results although a few were active after prolonged exposure to high concentrations. More recent results³¹ indicate that metabolic activation systems can be incorporated in this assay and make possible the detection of pro-carcinogens.

Mutation induction

Perhaps the most sensitive assay for DNA damage is the induction of mutations in bacteria, particularly if the bacterial strain carries a mutation rendering it unable to excise damage from DNA (Uvr⁻). Excision-proficient strains should always be included in any assay, however, because certain agents able to cross-link DNA are only mutagenic in such strains³²; presumably the mutational event occurs as an error during excision-initiated repair. Reversion to prototrophy is generally regarded as the most sensitive type of assay and the methodology has recently been reviewed^{33,34}. *Escherichia coli* WP2 is a tryptophan-requiring strain that responds to mutagens causing base-pair substitution mutations at both adenine:thymine and guanine:cytosine sites.

A more complete set of tester strains has been developed in *Salmonella typhimurium* by Ames and collaborators³⁵. Individual strains respond to base-pair substitution mutagens or to compounds causing various types of frameshift. The permeability of these *Salmonella* strains to some chemicals has been increased by the incorporation of a cell wall mutation ("deep rough")³². From recent data one can calculate that these strains are capable of detecting mutagenic activity of between 61%³⁵ and 90% (D. Anderson, unpublished) of known carcinogens. In an attempt to detect the "false negatives" obtained with the deep rough strains, Ames's group developed a fourth generation set of strains containing the drug resistance plasmid pKM101³³. As suggested by MacPhee certain plasmids confer a mutator activity on their host cell which becomes more sensitive to many mutagens and carcinogens³⁶. The ability of these plasmid-containing strains to detect carcinogens as mutagens is impressive (see below). A word of caution is in order, however, since although the mechanism by which the plasmids act is still unknown, it is clear that they convert into mutations damage which would not be mutagenic in a normal cell. They may even act as amplifying systems and produce mutations at sites where no damage exists. The value of such strains lies in the correlation they show with carcinogenicity but there is at least a theoretical possibility of real "false positives".

It is possible to improve sensitivity by altering the methodology, and a modified fluctuation test has been proposed which achieves between ten and one hundredfold greater sensitivity than the conventional assay without the need for plasmid-containing strains³⁷. There is also the possibility of developing a single tester strain that can be used to detect many different types of mutational events³⁸. Although bacterial screening systems have proved very useful, there is still scope for further improvement.

As well as the bacterial tests that have now been extensively studied, a large number of other techniques can be used to detect DNA damaging activity and may perform a useful supplementary role, probing ambiguous or suspect results and characterising more fully the nature of the genetic damage. One may, for example, study the induction of mutations in cultured mammalian cells³⁹⁻⁴¹. Mammalian cells may also be used for cytogenetic study of visible chromosome aberrations⁴². Recently developed staining techniques for demonstrating sister-chromatid exchanges show a greatly enhanced sensitivity⁴³ and their role in screening has been recently discussed⁴⁴. Sister-chromatid exchanges may now be detected in spermatogonia⁴⁵ and bone marrow cells⁴⁶ following exposure of the whole animal to carcinogens. It is already clear, however, that although the induction of sister-chromatid exchanges is a very sensitive response to some carcinogens it occurs hardly at all with others⁴³. Other eukaryotic mutation systems include fungi, yeasts and insects. All of these have their own advantages and disadvantages.

Malignant transformation in cultured cells

Rather than develop a model system depending on mutation or DNA repair, others have worked towards a screening method by which transformation to the malignant condition could be brought about and detected in cell culture. The only really valid criterion for malignant transformation is the ability of a cell to produce a tumour when inoculated into an appropriate host. There are, nevertheless, several secondary criteria (discussed by Freeman and Huebner⁴⁷, of which the most commonly used is the ability of cells to grow into clones in soft agar or, in the case of fibroblast cultures, to produce clones of piled-up cells when growing on a solid surface.

Most human cancers are carcinomas which are derived from epithelial cells. Relatively little work has, however, been done on the transformation *in vitro* of epithelial cells. Such cells are usually obtained from rat liver and are not easy to retain in culture in the differentiated state. Nevertheless they have been successfully transformed by 4-nitroquinoline-1-oxide⁴⁸, aflatoxin B₁, *N*-hydroxy-2-acetylaminofluorene, and 7,12-dimethylbenz[*a*]anthracene⁴⁹, dimethylnitrosamine and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine⁵⁰, and *N*-acetoxy-2-acetylaminofluorene⁵¹. Epithelial cells transformed *in vitro* usually show no altered morphology although they may grow in soft agar.

Work on fibroblasts is considerably more advanced. Fibroblasts, when transformed, give rise to sarcomas, responsible for a minority of human malignant disease. They show, nevertheless, great promise as the basis of a potential screening system for chemical carcinogens. As with bacterial mutation systems, it has often been found necessary to supplement the fibroblast's relatively poor ability to metabolise carcinogens into their active form. This has been achieved, either by cocultivation with other cells capable of carrying out metabolic activation⁵², or by isolating the cells from hamster embryos after treatment of the pregnant mother⁵³.

Much of the work on transformation has been carried out with treatment of mass cultures often for long periods of time and has been subject to some criticism. Transformation has, however, been reported with short treatments followed by cloning⁵⁴ and this would seem to be a

better approach to adopt in future. A noteworthy feature of much work with both fibroblast and epithelial systems has been the high spontaneous rates of transformation, sometimes considerably higher than one would expect for a gene mutation. Many workers regard this as the result of the artificial environment in which the cells are cultured, and have looked (often successfully) for conditions in which the spontaneous rate is lower. Nevertheless it is likely that the rate of transformation *in vivo* is higher than has been thought and that the body is normally able to deal effectively with the aberrant cells. If this were true the role of DNA-damaging carcinogens might be seen as increasing the already high spontaneous rate and thus overloading the ability of the natural defences of the body to cope with malignant cells.

Most of the work on transformation *in vitro* has concentrated on the development of systems that can be used as models for the study of carcinogenesis (for reviews see refs 55 and 56). To the uninvolved observer, a certain amount of contradiction and inconsistency is apparent. There is, notwithstanding, an impression that this technique may soon be a valued constituent of the battery of techniques for detecting carcinogenic and DNA-damaging substances. Two recent studies have shown successful prediction of carcinogenicity almost as good as that of the bacterial mutagenicity tests^{57,58}.

Validation

Some of the test procedures described above are very sensitive, but how good are they as predictors of carcinogenicity? Recent reports have presented⁵⁹ and discussed⁶⁰ carcinogenicity and mutagenicity data obtained for more than 300 chemicals. All the results come from the use of *Salmonella* strains developed by Ames. Where negative mutation results had been obtained with now-obsolete strains, the test was repeated with the latest plasmid-containing strains. Testing was done both without metabolic activation and (generally) with microsomes embedded together with the bacteria in soft agar. With some nitro-samines incubation was carried out with microsomes before plating. A summary of the results is given in Table 1. Since publication, one of the non-carcinogens, 5-hydroxy 2-acetylaminofluorene, has been shown to be mutagenic because of the presence of an impurity and has now been reclassified as non-mutagenic.

It can be seen that of 179 compounds whose carcinogenic effect on animals is well documented, 157 (or 87.7%) were detected as positive in the bacterial test. This level of confirmation was obtained with essentially all types of compound and was also evident for the small group of compounds for which evidence exists for carcinogenicity in man. The proportion of compounds believed to be non-carcinogenic which gave negative results in the mutagenicity tests was also high: 101 out of 117 (or 86.3%). These included 46 common biochemicals all of which were negative. Seventeen compounds were tested for which carcinogenicity data are uncertain; of these 11 were positive in the mutagenicity test.

The apparent "false" positives and negatives have been discussed elsewhere⁶¹. It is apparent that many of the latter damage DNA or cause mutations in other systems, or have mutagenic metabolites. Furthermore there is a very obvious limitation to the use of liver microsomes for activation: some chemicals may need reductive activation, or may be metabolised by the gut flora, by organs other than the liver, or by cell components other than microsomes. Indeed it is surprising that liver microsomes are as effective as they seem to be; certainly the method is capable of further improvement.

When one considers the "false" positives, that is the supposed non-carcinogens that register as mutagenic with bacteria, certain difficulties become apparent. Any correla-

Table 1 Correlation of animal carcinogenicity and bacterial mutagenicity with and without metabolic activation (from McCann *et al.*³⁵)

Group of compounds	Carcinogens detected as bacterial mutagens	Non-carcinogens not mutagenic to bacteria	Compounds of uncertain carcinogenicity detected as mutagens
A Aromatic amines etc.	23/25	10/12	5/7
B Alkyl halides, etc.	17/20	1/3	1/1
C Polycyclic aromatics	26/27	7/9	1/1
D Esters, epoxides, carbamates, etc.	13/18	5/9	0/1
E Nitro aromatics and heterocycles	28/28	1/4	0/2
F Miscellaneous organics	1/6	13/13	0/1
G Nitrosamines	20/21	2/2	1/1
H Fungal toxins and antibiotics	8/9	5/5	—
I Mixtures (cigarette smoke condensate)	1/1	—	—
J Miscellaneous heterocycles	1/4	7/7	—
K Miscellaneous nitrogen compounds	7/9	2/4	—
L Azo dyes and diazo compounds	11/11	2/3	3/3
M Common laboratory biochemicals	—	46/46	—
Total	157/178	101/117	11/17

tion is only as good as the confidence one has in the reliability of both parameters. Whereas positive and negative mutagenicity results can be both unambiguous and reproducible, the same is not true of carcinogenicity results where, as will be discussed below, there are several factors which could result in a failure to detect relatively weak carcinogens. As discussed by McCann and Ames³⁵, there is good reason to believe that many of the "false" positive chemicals will eventually be shown to be carcinogenic. This has already happened with the food additive furyl furamide which had been used for many years in Japan and had given negative results in two carcinogenicity trials³⁹. After positive results had been obtained in *Bacillus subtilis* and *E. coli* systems for detecting DNA damage, it was re-examined and shown to produce a low but significant yield for tumours when given to foetal and young mice⁴⁰. There is also the real possibility that some of the "false" negatives are genuine, that metabolism *in vivo* is different from that with isolated microsome preparations. Only further studies in depth can resolve this.

It is worth analysing the data of McCann *et al.*, further to see whether there is any particular type of mutational event (as detected by the *Salmonella*) that is correlated with carcinogenicity. It has been postulated⁶¹ that carcinogenicity is associated with the ability to produce specific types of frameshift mutation. This hypothesis does not hold up in any general application. As can be seen from Table 2, whereas most members of some groups of carcinogens (for example, aromatic amines, polycyclic aromatics and nitroaromatics) gave rise to both frameshifts and base-pair substitutions, others (for example, esters, epoxides and carbamates, nitrosamines, miscellaneous nitrogen compounds) gave rise exclusively to base-pair substitutions. There was no group that gave rise exclusively to frameshifts. Taken together, 45.2% of mutagenic carcinogens gave rise solely to base-pair substitutions, 14.8% solely to frameshifts, and 40% gave rise to both.

Rosenkranz (cited in ref. 62) using a Pol⁻ strain of *E. coli* together with the *Salmonella* set without plasmids, has obtained results as encouraging as those of McCann *et al.* with the plasmid-containing salmonellas. Of about 100 compounds tested, 85% of the known carcinogens were detected (91% of direct acting carcinogens, 72% of procarcinogens). The proportion of non-carcinogens detected as positive was rather high, 30%, but the figure is not comparable with the lower value derived from the data of McCann *et al.*³⁵ since it did not include the 46 common laboratory biochemicals tested by the latter workers, none of which was positive.

A comparison of the efficiency of various microbial systems for detecting DNA damaging agents has been carried out by Shirasu *et al.*⁶³. They found that the hypersensitivity of repair-deficient bacteria (Rec⁻ *B. subtilis*) was the most sensitive. Of 166 pesticides studied, 23 were posi-

tive in the Rec-assay (carried out without microsomal activation). Of the 143 negatives, none proved to be positive when tested with *E. coli* or *Salmonella* reverse mutation systems. Of the 23 positives 9 were positive in reverse mutation systems, and of these 9, 1 was not detected by the *E. coli* strains and 1 by the *Salmonella* strains. As far as base-pair substitution mutations are concerned, the non-plasmid *E. coli* strains were found to be preferable to the non-plasmid *Salmonella* strains at least with some groups such as nitrofurans. With other groups such as the organic phosphates a similar small proportion of mutagens was missed by both *S. typhimurium* and *E. coli* strains⁶⁴.

The only study in which a single laboratory has compared a number of different tests for predicting carcinogenicity appears to have been carried out by the Central Toxicology Laboratory of ICI (D. Anderson *et al.*, unpublished). The preliminary results with 120 chemicals point to the value of the bacterial mutation tests when metabolic activation is incorporated. The carcinogenicity, or non-carcinogenicity was accurately predicted for 90% of the chemicals by this test. Cell transformation *in vitro* came close with 83% accuracy. Rather less accurate was degranulation of endoplasmic reticulum, 72%, and morphological changes following subcutaneous implantation, 70% correctly predicted. Sebaceous gland suppression⁶⁵ was good for polycyclic hydrocarbons (90%) but little better than random for other substances (52–62%). Tetrazolium reduction in mouse skin was also poor (62% overall). The authors conclude that some of these rapid tests are capable of distinguishing between carcinogens and non-carcinogens with sufficient accuracy to enable them to be used for selecting potential carcinogens. They also make the point that figures for successful prediction must be treated with

Table 2 Number of carcinogens detected as bacterial mutagens (with or without metabolic activation) classified as to type of mutation induced.

	Base-pair substitutions only	Frameshifts only	Both base-pair substitution and frameshift mutations
A	1	9	13
B	14	2	1
C*	7	5	14
D	13	0	0
E	5	0	23
F	1	0	0
G*	19	0	0
H	0	3	5
I	0	1	0
J	0	1	0
K	7	0	0
L	3	2	6
M	0	0	0
Total	70	23	62

* Data not available for one member.

Key for chemical groups as for Table 1. (From McCann *et al.*³⁵)

some caution since they can be manipulated within wide limits by the choice of substances tested. As their substances include a large number of non-carcinogenic chemicals closely related to known carcinogens they feel that their results give a reasonably good indication of the likely value of the tests in practice.

DNA damage and human cancer

The correlation between mutagenicity and carcinogenicity is satisfying to those who believe in the somatic mutation theory of cancer⁶⁶ and distressing to those who do not⁶⁷. I think the correlation can be more correctly described as being between DNA damaging ability and carcinogenicity. Gene mutation is but one consequence of DNA damage; others such as chromosomal structural rearrangements, virus integration and excision, and changes in gene expression, may well be important in the carcinogenic process. Non-genetic effects are also probably involved.

One could argue that detecting DNA damage is merely a very sensitive way of detecting electrophilic reagents, and that the actual target(s) may well be in other molecules as well as or instead of DNA. This is quite possible; but there is other evidence strongly implicating DNA damage as the rate-limiting step in many carcinogenic processes.

In man, for example, mutations in five complementation groups are known to reduce or abolish the ability of cells to remove ultraviolet photoproducts from their DNA⁶⁸. In all cases they enormously increase sensitivity to the carcinogenic effect of sunlight (resulting in the hereditary disease xeroderma pigmentosum). A further mutation causing the same symptoms has been shown to be associated with a deficiency in another DNA repair pathway active on newly synthesised DNA⁶⁹. Another human mutation responsible for the disease ataxia telangiectasia has been shown to block repair of ionising radiation damage⁷⁰ and also results in proneness to develop malignant disease⁷¹. Thus, the human data strengthen our confidence in the reality of the observed correlation between DNA damaging ability and carcinogenicity. It must be emphasised, however, that even an empirical "litmus paper test", with no known theoretical basis, which gave an 80 to 90% predictiveness for carcinogenicity would be a powerful tool in the screening of chemicals for human toxicity.

The place of tests with mammals

No single test is adequate for a first-tier (sub-mammalian) screen; most authorities agree that a battery of tests must be used as false negatives may occur with any one test. The results of these tests would be used to assign priorities for further testing using mammalian systems. At one extreme, a substance with no apparent effect on sub-mammalian systems might be given a priority so low that no further tests would be considered unless a large human population exposure were to occur or be contemplated. At the other extreme a strongly active substance might well be regarded as hazardous without further testing if the population exposed were small. If it were, say, an industrial chemical, then production workers and users ought to treat it as if it were a known toxic agent or carcinogen, at least until such time as it became possible to carry out full scale animal tests.

The greatest problem in testing for carcinogenicity or mutagenicity with mammals is the insensitivity of most of the tests. This has led, for example, to difficulties in validating microbial carcinogenicity screening systems since many of the "false" positives obtained with these are based on animal experiments that may be inadequate⁶⁸. There have been, and still are, too many carcinogenicity tests with 20 or 30 animals per group.

Provided that the number of animals in each group is kept small, even a large increase in the frequency of neo-

plasms can fail to be statistically significant and enable a conclusion of "non-carcinogenic" to be drawn (see for example a recent study on the carcinogenicity of hair dyes⁷²). As long ago as 1954, Barnes and Denz⁷³ pointed out that to detect with a probability of 0.01 an effect occurring in 1% of the animals, one would need a group of at least 455 animals. If the effect also occurred spontaneously then the number of animals per group would have to be increased manyfold. Today, notwithstanding, carcinogenicity experiments with 100 animals per group are often regarded as "good" and those with 200 animals per group are extremely rare. But although "kilomouse" experiments are theoretically attractive there may be little to be gained from them in practice. Logistical problems dictate that such experiments be phased over many weeks and involve slightly varying conditions. "Spontaneous" rates of tumour occurrence unfortunately often vary in time and place, perhaps reflecting slight differences in diet, and it is often difficult to run an adequate control group. Errors in handling are also more likely in very large experiments.

Whereas a significant reproducible positive result in a mammalian test may be taken as indicating the existence of a potential hazard for man, a negative result taken should not necessarily be taken to indicate the absence of hazard, particularly if the human population to be exposed is very large, and the number of animals in the test small. We may take some comfort where the disparity in dose between the animal and human exposure is great. This is not always so. Anaesthetic gases, for example, are given to an appreciable fraction of the population in Western society at concentrations which are not far from the lethal level. One might well feel that a negative result in a screening test with a few dozen mice would be of little value.

It can be seen that mammalian tests are not wholly appropriate for the validation of sub-mammalian tests, and it is perhaps remarkable that they should show such good agreement. Validation of one type of test against another must not blind one to the real objective, which is to predict long term toxic effects in man. There are few proven human carcinogens and most of these can be detected by both mammalian and sub-mammalian tests. In man, carcinogens tend to be recognised only when the tumour is of a rare type and there is a sufficient cluster of cases to enable association with a particular occupation to be seen by an alert clinician. Genetic effects in man are even harder to detect retrospectively. There are several examples of somatic chromosome damage in lymphocytes of persons exposed to known mutagenic and carcinogenic substances (for example, vinyl chloride⁷⁴, ozone⁷⁵, benzene⁷⁶, toluene⁷⁷, cadmium^{78,79} and methyl mercury⁸⁰). Recent evidence for the possibility of dominant lethal damage in man by vinyl chloride (P. Infante, unpublished) and anaesthetic gases⁸¹ is ominous but needs closer examination.

Ultimately quantitative risk assessments must be attempted, for we must face the unpalatable fact that man will almost certainly have to be exposed to some carcinogens and mutagens whose benefits cannot be dispensed with and others which it is impracticable to eliminate from the environment. Risk evaluations at the present time almost always require information which is not available, such as the nature of the dose-effect response at low doses. Nevertheless, approaches must be found that will lead eventually to risk-benefit evaluations based less on guesswork and more upon knowledge. In the meantime the use of short term tests would enable potentially carcinogenic substances to be identified among the many thousands for which long term animal testing cannot at present be contemplated. This in turn would open the door to provisional regulatory action to minimise human exposure. Taken seriously and on a large enough scale, there is good reason to believe that this approach would ultimately result in a reduction in the incidence of chemically induced cancer.

I thank Drs D. Anderson, E. J. Ashby, P. A. Lefèvre, E. Longstaff, I. F. H. Purchase, J. A. Styles and F. R. Westwood of ICI Central Toxicology Laboratory, and Dr Bruce Ames for allowing me to see their data before publication.

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articles

Isolation and N-terminal amino acid sequence of membrane-bound human HLA-A and HLA-B antigens

John Bridgen

Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

David Snary & Michael J. Crumpton*

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

Colin Barnstable, Peter Goodfellow & Walter F. Bodmer

Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

Membrane-bound HLA-A and HLA-B antigens have been extensively purified in good yield. The sequences of the N-terminal 16 amino acids have been determined using about 1 nmol of protein eluted from polyacrylamide gel after electrophoresis in sodium dodecyl sulphate.

THE major histocompatibility region of man (HLA) occupies at least 1 to 2 recombination units on chromosome 6 and probably contains a large number of genes involved in diverse immune and possibly other functions¹. The gene products that have so far been identified with this region comprise various

*Reprint requests to M. J. Crumpton at the above address.

Table 1 Purification of HLA-A2 antigen from BRI 8 cells

Fraction	Protein (% recovery)	HLA-A2 activity (% recovery)	Degree of purification of HLA-A2
BRI 8 cells	100	100	1
Plasma membrane	1.0	46	45
Na deoxycholate-soluble	0.95	38.8	41
Gel-filtration	0.26	36.1	136
Glycoprotein	0.026	32.5	1,240

BRI 8 cells grown by Searle Diagnostic were used as the antigen source. The cells (about 80% viable; 5×10^7 cells ml⁻¹ of 10 mM Tris-HCl buffer-0.15 M NaCl, pH 7.4) were broken using the Stansted cell disruptor (model A0 612; disrupting valve 516; air pressure applied to disrupting valve 12 p.s.i.). The plasma membrane fraction was separated, as previously described¹⁵, by differential centrifugation and discontinuous sucrose density gradient centrifugation (36% (w/v) against 25 (w/v) sucrose as judged by refractive index measurement), and was washed by homogenising twice in 10 mM Tris-HCl buffer, pH 7.3. The membrane (4 mg of protein ml⁻¹) was solubilised at 0 °C by addition of sodium deoxycholate (10% (w/v) in 10 mM Tris-HCl buffer, pH 8.2) to a final concentration of 2% and was immediately centrifuged at 100,000g for 1 h. The supernatant (7 ml) was subject to gel filtration on a column (55 × 5.0 cm) of Ultragel AcA 34 (LKB Instruments) in 0.5% deoxycholate at a flow rate of 70 ml h⁻¹. The fractions corresponding to the A and B antigens (see Fig. 2, ref. 16) were pooled (about 60 ml) and added to a column (16 × 1.6 cm) of *L. culinaris* lectin-Sepharose 4B (1 mg of lectin per ml of gel sediment) in 0.5% Na deoxycholate. The bound glycoproteins were subsequently eluted with 2% methyl- α -D-mannopyranoside in 0.5% Na deoxycholate (see Fig. 2, ref. 15). The results shown were obtained using lectin isolated from lentils purchased from Whitworths. Similar results have been consistently obtained using lentils from this source, but a few batches of lentils from other sources have given lectin that bound a portion of the HLA-A and -B activities only. A, B and C antigen activities were measured by inhibition of cytotoxicity¹⁸ using antisera which have been previously described¹⁶. The purification of the A1, B8 and B13 antigens was similar to that illustrated for the A2 antigen.

cell surface antigenic determinants as well as the C2 (ref. 2), C'3 proactivator³, C4 (ref. 4) and C8 (ref. 5) components of complement. The most extensively characterised cell surface antigens are those of the three highly polymorphic segregant series of the HLA-A, -B and -C loci¹⁻⁶. The HLA region also codes for a set of serological specificities, called Ia (immune-associated) antigens⁷. These probably represent the products of several loci (refs 7-10 and J. G. Bodmer, personal communication), some of which show a close association with the HLA-D locus that controls the expression of the major determinants involved in mixed lymphocyte responses¹¹. The functions of these loci have yet to be delineated but they seem to be connected with the ability to generate immune responses against foreign substances¹², tumours and virus-infected cells¹³ as well as other phenomena related to cell-cell recognition. A complete understanding of the biological relevance of these gene products relies ultimately upon their isolation in a highly purified state and the determination of their structure.

The choice of tissue for the isolation and subsequent structural studies of the HLA-A, -B, -C and Ia antigens has been dictated by several considerations. HLA-A, -B and probably -C locus antigens are expressed on the surface of almost all cell types except erythrocytes. Ia antigens, however, show a much more restricted tissue distribution, being found on B lymphocytes, monocytes and possibly some endothelial and epithelial cells, but not T lymphocytes and fibroblasts¹⁴. Our choice of cultured human lymphoblastoid B cell lines as the tissue source has not only enabled the simultaneous purification of the HLA-A, -B and Ia antigens, but has also permitted the purification of antigen of restricted and reproducible specificity in amounts sufficient for structural studies.

The selection of purification procedure was influenced by various factors such as HLA-A and -B; Ia antigens are integral plasma membrane glycoproteins and being insoluble in conventional aqueous solvents need to be solubilised in detergents¹⁶. In particular, the fractionation techniques were selected because they gave a high yield coupled with considerable discrimination. This was necessary because of the high cost of cultured cells and because the HLA-A and -B antigens comprise <0.1% of the total cell protein. The separation of the cell surface (plasma) membrane was chosen as the initial purification step in preference to either the preparation of a crude membrane fraction or the extraction of whole cells with detergent. The reasons for choosing this approach were that it greatly reduced the possibility of degradation and of the generation of structural artefacts, such as dimerisation through disulphide interchange¹⁸, as well as giving a significant degree of purification. The success of this approach depends, however, on being able to prepare highly purified plasma membrane in good

yield (preferably better than 50%).

The structural analysis was facilitated by the availability of a new microtechnique for determining N-terminal amino acid sequences (J. B. unpublished). This technique combines high sensitivity with the considerable resolving power of SDS-polyacrylamide gel electrophoresis, and is applicable to less than 1 nmol of protein eluted from polyacrylamide gels.

Purification of HLA-A and -B antigens

The purification of HLA-A2 antigen from the lymphoblastoid B cell line BRI 8 (HLA specificities: A1, A2, B8, B13 and CW2) is summarised in Table 1. The A1, B8 and B13 antigens co-fractionated with the A2 antigen and were purified to

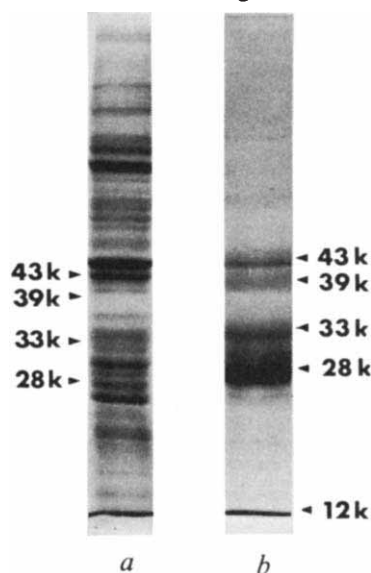


Fig. 1 Polyacrylamide gel electrophoresis in SDS. *a*, BRI 8 plasma membrane; *b*, glycoprotein fraction (Table 1). Electrophoresis was performed in 10% (w/v) polyacrylamide slab gels using the Tris-glycine discontinuous buffer system of Laemmli²¹. Samples were dissolved by heating at 100 °C for 2 min in 2% (w/v) SDS, 0.1 M dithiothreitol, 10% (w/v) glycerol and 0.02% bromophenol blue. Gels were stained with 0.1% Coomassie blue in methanol, water, acetic acid (41:52:7, by volume) and destained with the same solvent. Molecular weights were calculated by reference to the mobilities of standard proteins: transferrin (78,000), bovine serum albumin (69,000), ovalbumin (45,000), immunoglobulin L chain (25,000), soybean trypsin inhibitor (21,000) and cytochrome *c* (12,500). In the above conditions β_2 -microglobulin was coincident with the bromophenol blue band at the gel front. The presence in the glycoprotein fraction of a band with a molecular weight of 12,000 was revealed by using 12% (w/v) polyacrylamide gels.

similar extents. The yield and purity of the purified plasma membrane is intimately related to the method used for cellular disruption¹⁷. In this study the cells were broken using a commercial model of the cell-disrupting pump (Stansted Fluid Power). The plasma membrane was separated by differential centrifugation and discontinuous sucrose density gradient centrifugation¹⁸ and was washed by homogenising under hypotonic conditions. Under these conditions yields of purified plasma membrane in excess of 50% are often achieved. The purified membrane was solubilised in sodium deoxycholate before purifying the A and B antigens by conventional separation techniques such as gel filtration and affinity chromatography. Sodium deoxycholate (final concentration 2%) proved superior as the solubilising agent to other weakly anionic detergents such as Ammonyx Lo, and the non-ionic detergents Nonidet P-40 and Triton X-100. It was selected in preference to the strongly ionic detergents such as SDS because, in contrast to these detergents, it had a negligible effect on antigenicity and did not interfere with specific molecular interactions¹⁹. The solubilised membrane was fractionated by gel filtration on Ultragel AcA 34 in sodium deoxycholate. Ultragel AcA 34 was used in preference to Sephadex G-200 and Bio-Gel P-150 because of its superior column stability and flow rate. The glycoproteins of the gel filtration fraction were next separated from the non-glycosylated proteins by selective adsorption to *Lens culinaris* lectin-Sepharose 4B in sodium deoxycholate and subsequent elution with methyl- α -D-mannopyranoside. Gel filtration was selected to precede affinity chromatography since adsorption to and elution from *L. culinaris* lectin-Sepharose provided a very convenient concentration step. As shown in Table 1 the overall purification procedure gave a 1,240-fold increase in the specific activity of the A2 antigen relative to BRI 8 cells with a recovery of 33%.

The CW2 antigenic activity of BRI 8 cells co-fractionated

with the A and B antigens up to the gel filtration step but was not detected in the glycoprotein fraction. The results suggest that it was not adsorbed to *L. culinaris* lectin-Sepharose due either to a different carbohydrate composition (compare the behaviour of rat thymocyte Thy-1 antigen in ref. 20) or to the absence of carbohydrate.

The glycoprotein fraction was recovered by precipitation at -20°C for 48 h with 66% (v/v) ethanol in which deoxycholate and sugar are soluble, and was analysed by polyacrylamide gel electrophoresis in SDS. Figure 1 shows that the glycoprotein fraction has a very restricted polypeptide composition compared with that of the original plasma membrane preparation. Only five bands whose position corresponded to molecular weights of 43,000, 39,000, 33,000, 28,000 and 12,000 were detected. Various results indicate that the 43,000 and 12,000 molecular weight chains are derived from the HLA-A and -B antigens. This conclusion is based on the reports²²⁻²⁴ that the A and B antigens are composed of two non-covalently-bound polypeptide chains with molecular weights of about 43,000 and 12,000, and that the smaller chain is identical with β_2 -microglobulin. It is also supported by the observation that only these two chains were specifically adsorbed when the glycoprotein fraction (Table 1) was added to a column of antibody to β_2 -microglobulin attached to Sepharose and were subsequently eluted by 3 M potassium thiocyanate in 0.5% sodium deoxycholate, pH 8.5 (ref. 25).

The remaining polypeptide chains are of unknown function, although the following evidence suggests that those of molecular weight 33,000 and 28,000 represent the human counterparts of the mouse Ia antigens. First, the polypeptide chains of mouse Ia antigens possess similar molecular weights of about 30,000 (ref. 26). Second, the Ia allogeneic activity expressed on the surface of BRI 8 cells co-fractionated with the A and B antigens on gel filtration and *L. culinaris* lectin-Sepharose²⁷. Third,

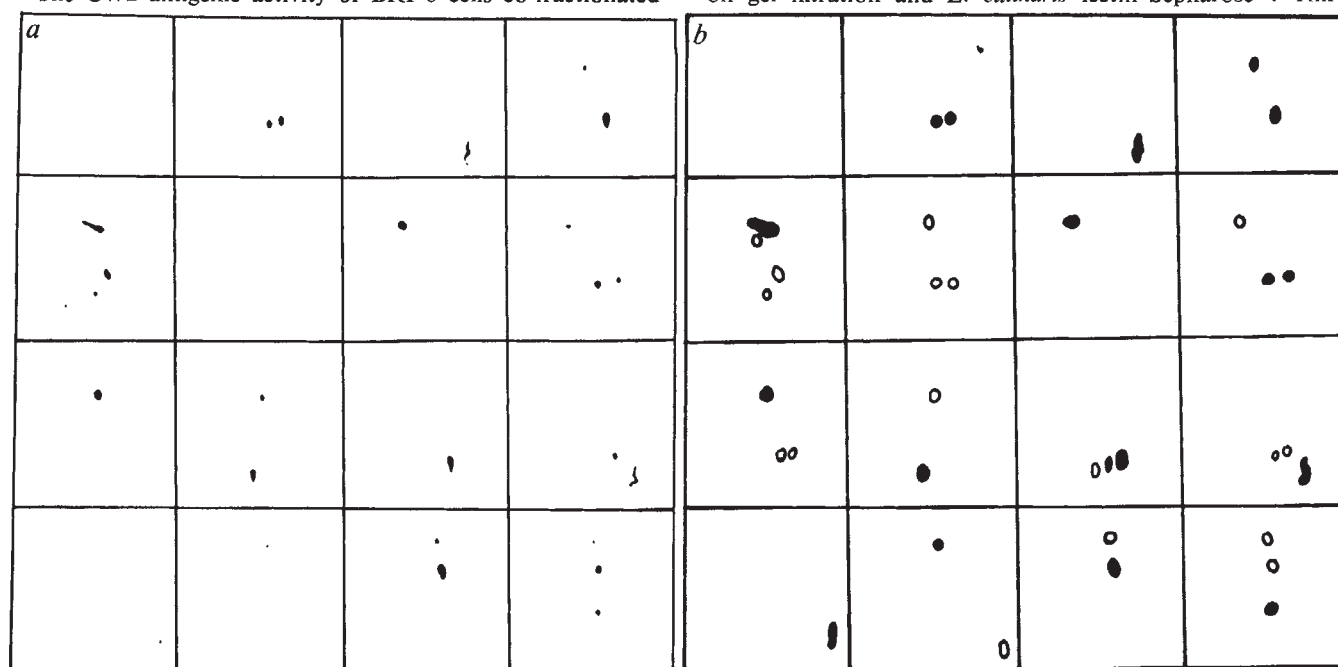


Fig. 2 *a*, Autoradiograph of thin-layer chromatograms resulting from sequence analysis of the 12,000 molecular weight polypeptide chain recovered after polyacrylamide gel electrophoresis of the glycoprotein fraction in sodium dodecylsulphate (SDS). Phenylthiohydantoin-amino acids were separated on 5×5 cm polyamide layers by 2-dimensional chromatography²³ using 5-bis [2(5-*tert*-butylbenzoxazolyl)]-thiophene (0.1%) as fluorescent indicator²⁴. The phenylthiohydantoin of histidine and arginine were resolved by redeveloping the chromatograms in the direction of the first solvent using *n*-propanol, formic acid (20:1, v/v). Radioactive spots were revealed by autoradiography using Kodak 'Auto-process' X-ray film and were identified relative to the unlabelled internal markers that were visualised under ultraviolet light. The autoradiograph was exposed for 60 h. The variation in intensity of the spots at successive steps partly reflects the varied amount of cyclised derivatives analysed. The residues are arranged in sequence from left to right. The sequence shown begins at residue no. 2 since residue no. 1 remains attached to the resin and is not identified. The blank at residue no. 6 indicates that no information is available on this residue although, by implication, it is lysine which would remain attached to the resin and consequently would not be identified. The origin of the background of faint spots that increased in intensity with increase in the number of steps is not clear. This background was independent of the protein analysed (see Fig. 3). In the above conditions phenylthiohydantoin-glutamine (residues nos 2 and 8) and phenylthiohydantoin-serine (residue no. 11) give two spots due to decomposition (see also Fig. 3, residues nos 2 and 4). *b*, Drawing of autoradiograph. The filled-in areas correspond to the spot(s) due to the amino acid(s) identified at each step. The open circles represent spots which were detected but which were judged to be due either to overlap from the previous residue(s) or to background.

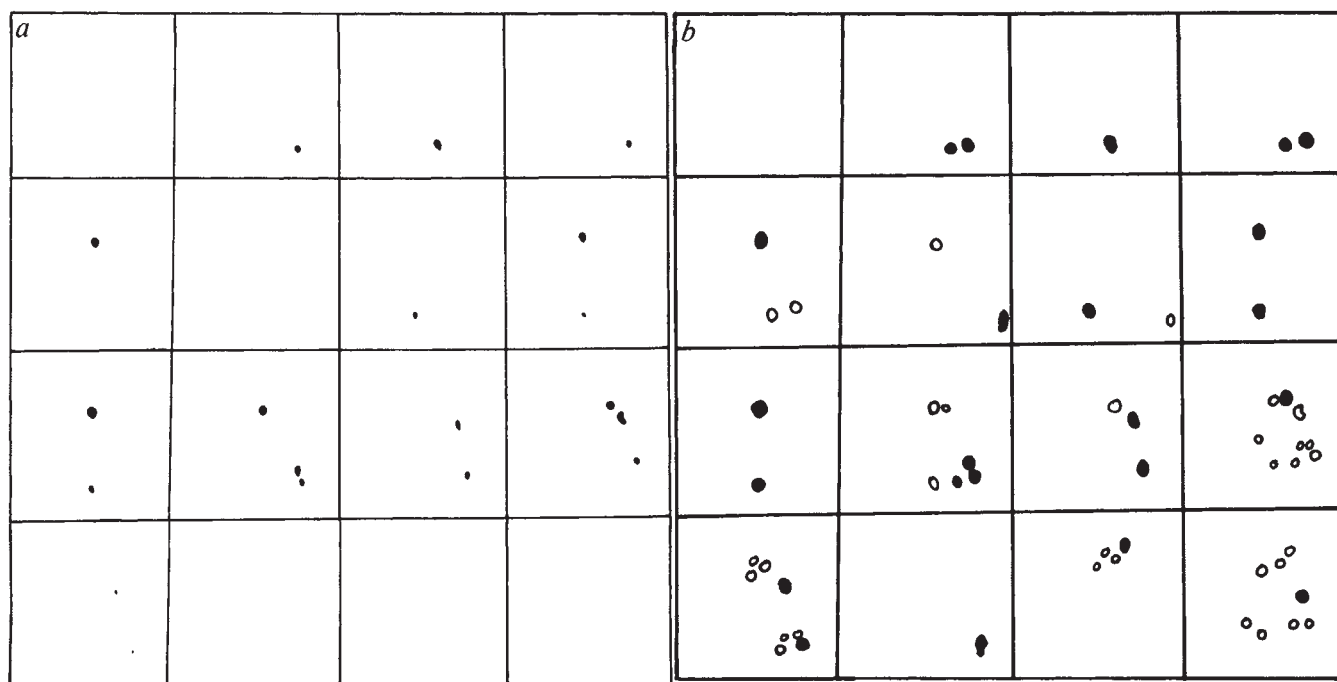


Fig. 3 a, Autoradiograph of thin-layer chromatograms resulting from sequence analysis of the 43,000 molecular weight polypeptide chain recovered after polyacrylamide gel electrophoresis of the glycoprotein fraction in SDS. Experimental details were as described in the legend to Fig. 2 except that the autoradiograph of the first twelve residues was exposed for 60 h. and that of residues nos 13–16 for 120 h. The sequence determined from the data is shown in Table 2. **b**, See Fig. 2b legend.

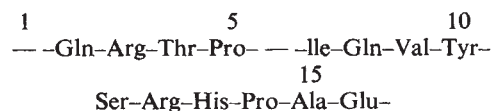
antisera produced in rabbits and mice against the 33,000 and 28,000 polypeptide chains of the glycoprotein fraction had many of the properties expected for heterologous anti-Ia sera^{27,28}. Thus, they reacted preferentially with human B-lymphoblastoid cell lines, purified peripheral blood B lymphocytes and monocytes, but not T cell lines, peripheral T cells, fibroblasts and erythrocytes. The immunoglobulin fraction of the antisera and its Fab fragments also blocked human mixed lymphocyte cultures²⁷.

Amino acid sequence of A and B antigens

The N-terminal amino acid sequences of the 43,000 and 12,000 molecular weight chains were determined by the high-sensitivity solid-phase procedure²⁹ using material extracted from the polyacrylamide gel (J. B., unpublished). As soluble foreign material occluded in the gel, and the glycine of the Laemmli buffer system²¹ interfered with the subsequent procedure, the following separation method was adopted. The polyacrylamide gel (15%, w/v) slab (8 × 7 × 0.15 cm) was washed overnight in 0.17 M Tris-HCl buffer, pH 8.15, containing 0.1% SDS, reinserted into the gel apparatus and cut to size before casting a 3% (w/v) polyacrylamide stacking gel on to the top edge. The glycoprotein fraction (400 µl containing 100 µg of A and B antigens in 2% SDS, 0.1 M dithiothreitol, 10% glycerol and 0.02% bromophenol blue) was added and electrophoresis was carried out using the Trisborate discontinuous buffer system of Neville³⁰. The edges of the gel were detached, stained for 30 min with 0.1% Coomassie blue and destained for 90 min in methanol–water–acetic acid (41:52:7, by volume) followed by 10 min in 7% (v/v) acetic acid. Using the stained strips as guides the regions corresponding to the 43,000 and 12,000 molecular weight chains were removed, cut into 2-mm cubes and extracted overnight by shaking at 37 °C in 1 ml of 0.04 M *N,N'*-dimethyl-*N*-allylamine buffer in pyridine, water (3:2, v/v) (Pierce Chemical Co.) containing 0.1% SDS and buffered to pH 9.5 with trifluoroacetic acid. The eluted protein was covalently attached to *N*-(2-aminoethyl)-3-aminopropyl glass (120 Å pore diameter) that had been activated by treatment with phenylene diisothiocyanate in dimethylformamide²⁹.

Amino acid sequences were determined on an Anachem SPA 1200 automatic solid-phase sequencer using high specific activity ³⁵S-labelled phenylisothiocyanate (2% (v/v) solution in acetonitrile containing 1 mCi ml⁻¹; Radiochemical Centre, Amersham) for the coupling. The reaction was completed by a second coupling with unlabelled phenylisothiocyanate (5% solution in acetonitrile). The cleaved thiazolinones were collected into tubes containing all the conventional phenylthiohydantoin-amino acids (1 nmol of each in 10 µg of ethyl acetate) to act as carrier and markers. The thiazolinones were converted to thiohydantoin by heating at 80 °C for 10 min in 200 µl of 20% aqueous heptafluorobutyric acid. The phenylthiohydantoin-amino acids were extracted with ethyl acetate and were identified by two-dimensional thin-layer chromatography.

The applicability of the method is evident from Fig. 2 which shows the autoradiograph obtained for the N-terminal 16 residues of the 12,000 molecular weight polypeptide chain of the glycoprotein fraction. The amino acid sequence is clearly discernible as:



This sequence is, apart from the blanks at residues no. 1 and 6, identical with that determined previously by Cunningham and coworkers³³ using conventional techniques, for the N-terminus of human urinary β_2 -microglobulin.

The autoradiograph of the N-terminal 16 residues of the 43,000 molecular weight polypeptide chain is shown in Fig. 3. The identification of the spots was straightforward apart from that of residue 3 which could not be related to any known phenylthiohydantoin-amino acid; this residue was also not identified by conventional sequencing methods (Table 2). One possible explanation is that this residue is substituted by carbohydrate. The interpretation of the sequence was less straightforward than that of β_2 -microglobulin. This was due to the heterogeneity of the 43,000 molecular weight polypeptide

Table 2 Comparison of N-terminal sequences

Protein	Antigenic specificity	Residue no.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
43,000 molecular weight chain	A1, A2	}	Ser		Ser	Met	Arg	Tyr	Phe	Phe	Thr	Ala	Val	Ala	Arg	Pro	Gly
	B8, B13								Tyr	Tyr	Ser	Ser		Ser			
Papain-solubilised 34,000 molecular weight chain	A2	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Phe	Thr	Ser	Val	Ser			Gly
	B7, B12	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Tyr	Thr	Ala	Val	Ser	Arg	Pro	Gly

Amino acid sequence of the N-terminal 16 residues of the 43,000 molecular weight polypeptide chain compared with the N-terminal sequences of the larger polypeptide chains of the papain-solubilised HLA-A2, and mixed HLA-B7 and HLA-B12 antigens reported by Terhorst and coworkers³⁴. The A and B antigenic specificities of the 43,000 molecular weight polypeptide chain are based on the known specificities of the glycoprotein fraction and on reports that the allogeneic A and B specificities are associated exclusively with a polypeptide of this molecular weight²⁴. The sequence of the 43,000 molecular weight chain is based on the results shown in Fig. 3 and begins at residue 2 since residue 1 remains attached to the resin and is not identified. Blank positions indicate that no information is available on the residue at this point. Residue 3 of the 43,000 molecular weight chain gave a detectable spot on thin-layer chromatography (Fig. 3) but this spot could not be identified with any known phenylthiohydantoin-amino acid.

chain (A and B antigens with polymorphic specificities) and to the need to differentiate between the presence of more than one residue at a particular position and overlap from the previous residue, especially when the same amino acid occurred at adjacent positions (residues 8 and 9, for example). Overlap was judged on the basis of experience gained from the analysis of unique sequences (J. B., unpublished). The most probable sequences are presented in Table 2 together with the N-terminal sequence of the papain-solubilised HLA-A2, and mixed HLA-B7 and HLA-B12 antigens determined using conventional methods³⁴.

It is apparent that the solid-phase sequencing method has much to recommend it. It is applicable to <1 nmol of protein eluted from polyacrylamide gel after electrophoresis in SDS and is rapid (1 cycle (residue) h⁻¹). As with conventional methods the extent of sequence attainable is limited by decrease in yield, increase in background and overlap from the previous residue. The sequence of β_2 -microglobulin could have been extended beyond residue 16, but little further information was obtainable from the 43,000 molecular weight chain.

Polymorphism

Clearly the N-terminal sequence of the 43,000 molecular weight polypeptide chain represents the sum of the sequences of the papain-solubilised A and B antigens, apart from some slight variation at residues 6, 8, 10 and 13. The marked similarity between the N-terminal sequences indicates that papain must cleave the polypeptide chain towards the C-terminus and that the N-terminus is exposed on the cell surface. This means that the portion of the polypeptide chain inserted into the lipid bilayer of the membrane is close to the C-terminus, although it does not rule out the possibility that the actual C-terminus is exposed on the inner or outer face of the membrane. The same conclusions have been drawn for the homologous mouse H-2 antigens³⁵. Similarly, it is apparent from the identity of the N-terminal sequences of the membrane-associated and urinary β_2 -microglobulins, that if the urinary material has been degraded then cleavage must have occurred towards the C-terminus.

If, as seems likely, the HLA-C locus products were not present in the material used for sequence analysis, then several interesting points emerge from the data in Table 2. First, at least 10 of the N-terminal 16 residues are apparently constant for both the A and B locus antigens, and all of the amino acid variations correspond to single nucleotide base changes. This restricted heterogeneity is consistent with the suggestion that the A and B genes arose from a common ancestral gene by duplication^{1,36}. In this context the sequence of the C antigen will be particularly informative since, although its molecular size and shape in deoxycholate resemble more closely those of the A than the B antigens (ref. 16 and C.B., W.F.B., M.J.C.,

and D.S., unpublished), the strong linkage disequilibrium between the C and B locus determinants suggest that the C gene(s) arose from the B gene(s)³⁶. Second, the sequence differences at residues 9 and 11 can be interpreted as A and B locus differences, but those at residues 6, 10 and 13 cannot be interpreted in this way. Thus, the A1, A2, B8 and B13 antigens collectively have arginine at residue 6 whereas the B7 and B12 antigens have arginine and valine. This variation is consistent with the suggestion that the B7 or B12 antigen differs from other A and B antigens in having valine at this position. Similarly, the serine and alanine residues at positions 10 and 13 respectively may represent changes that are directly related to the A1, B8 and/or B13 specificities. If these and other changes do contribute to the serologically defined polymorphism then each allele may represent the sum of a number of changes at positions remote from each other in the unfolded polypeptide chain. The present data are too limited to provide an answer to this proposal or to the postulated common origins of histocompatibility antigens and immunoglobulins^{1,37}. Definitive answers will, however, emerge as more sequence data are accumulated. It will, in general, be interesting to delineate the evolutionary relationships between the products of the different alleles and loci of the HLA system, and, in particular, to see whether cross-reacting antigens (A2 and A28, for example) have some common changes that distinguish them from all other antigens as well as additional changes that distinguish them from each other.

Received March 22; accepted April 14, 1976.

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Cell-cycle, cell-shape mutant with features of the G₀ state

M. St. J. Crane & D. B. Thomas

Immunology and Experimental Biology Division, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

A cold-sensitive mutant of CHO cells has features of "reverse transformation" at the non-permissive temperature of 33 °C. Cells accumulate at G₁ with altered morphology and remain viable and quiescent for more than 40 d. Such cultures are synchronised by a temperature shift back to the permissive 39 °C.

THE selection and isolation of temperature-sensitive mutants of eukaryotic cells defective at a specific phase of the cell division cycle (*cdc*) provides an attractive model for the study of growth control *in vitro*. Hartwell *et al.*^{1,2} have pioneered this approach by isolating 150 *cdc* mutants of the yeast *Saccharomyces cerevisiae* defective in the initiation of DNA synthesis, DNA replication, bud emergence and cytokinesis. More recently, heat-sensitive mutants of mammalian cells have been described including variants defective at metaphase³, G₁^{4–6}, DNA replication^{7,8}, and cytokinesis⁹. Most mammalian *cdc* mutants, however, are disappointing in two important respects: rapid loss of viability at the non-permissive temperature and failure to be synchronised by a temperature shift.

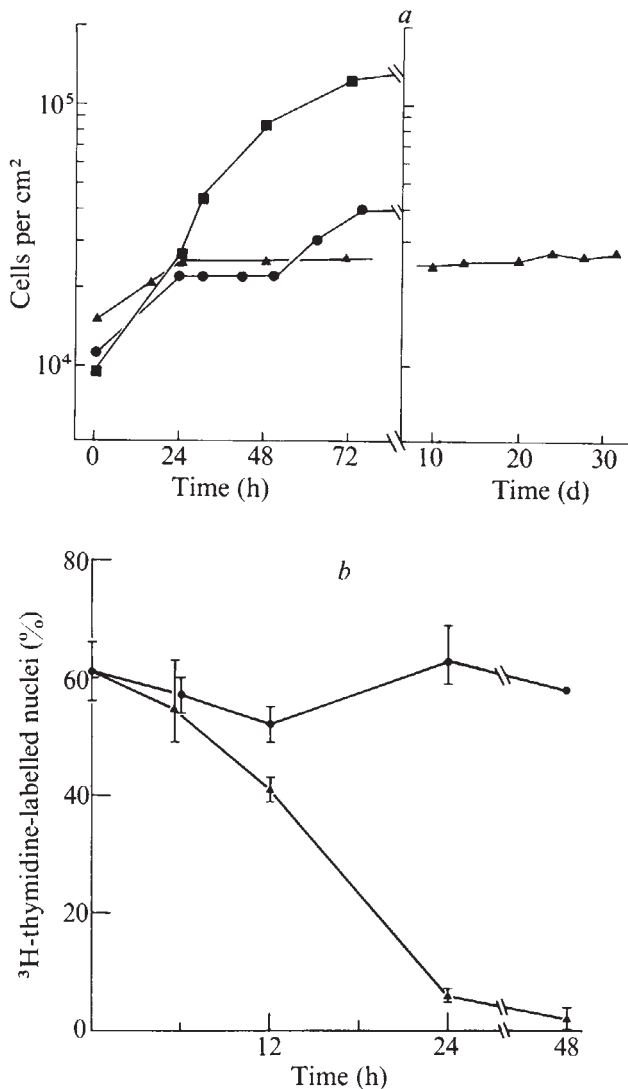
We describe here a cold-sensitive mutant of Chinese hamster ovary cells, clone cs4-D3 initially isolated and provided by Dr Rosann Farber, which has unique features at the non-permissive temperature (33 °C). First, cells accumulate at the G₁ interval and remain viable and quiescent indefinitely. So far, we have maintained the mutant at 33 °C for 40 d without loss of viability or potential for growth at the permissive 39 °C. Second, there is a cell-cycle-dependent change in morphology: cells are transformed from their normal "fibroblast-like" shape to a round, epithelial form. This shape change can be reversed at the non-permissive temperature by addition of dibutyl (db) cyclic AMP. Finally, cells can be synchronised by a temperature shift (39 °C to 33 °C to 39 °C) and then undergo several rounds of synchronous division. This is the first report of a cell cycle mutant reversibly arrested in a quiescent state. Clone cs4-D3 may provide a useful *in vitro* model for the study of the induction of DNA synthesis and the relationship between cell morphology and cell growth.

Selection and isolation of cold-sensitive mutants were as reported before¹⁰. Briefly, CHO cells were mutagenised with ethyl methane sulphonate and selected at 33 °C by the BUdR-visible light technique. Stock cultures of cs4-D3 were maintained at 39 °C as monolayers (Falcon culture flasks; 25 cm² surface area) in Eagle's basal diploid medium—10%

foetal calf serum supplemented with non-essential amino acids and passaged twice weekly at a split ratio of 1:20. Experimental cultures were established in disposable glass scintillation vials (surface area 4.3 cm²; 2–4 × 10⁴ cells) and maintained at a constant temperature (± 0.1 °C) in a water bath fitted with a contact thermometer (Grant Instruments, Cambridge Ltd).

When exponentially growing cultures of cs4-D3 were stepped down to the non-permissive temperature (39–33 °C) cell numbers increased by about one population doubling to plateau values (Fig. 1a; division index, 0.6–0.9) and the number of ³H-thymidine-labelled nuclei fell to base line (<2% at 48 h; Fig. 1b), while samples established at a similar density from stationary cultures increased no further in cell number. In contrast, growth of wild-type CHO cells was only marginally reduced at 33 °C (Fig. 1c). cs4-D3 remained viable at the non-permissive temperature for a minimum of 40 d, as determined by dye exclusion tests (>95%) and showed no sign of detachment from the glass surface. Moreover, cells retained the ability to grow at the permissive temperature (see below and Fig. 3). In long term experiments (>5 d) this was routinely tested at 2-d intervals by "stepping up" replicate cultures and measuring increases in cell number. The mutant was "leaky" at the slightly higher temperature of 34 °C and, after an initial growth plateau, there was a further increase in cell number by 72 h (Fig. 1a). This emphasised a need for strict temperature control (33 ± 0.1 °C) of all experimental cultures.

Change in morphology was perhaps the most striking feature of this mutant. At the permissive temperature, cs4-D3 resembled a fibroblast with an elongate, spindle shape and smooth surface membrane (Fig. 2a) whereas at 33 °C most cells (>90% by 24 h) were round and epithelial-like with a rough surface membrane bearing knob-like protrusions (Fig. 2b). Moreover, the epithelial form assumed by the mutant at the non-permissive temperature could be reversed by the addition of db cyclic AMP (Fig. 2c). This is shown in the following experiment. Coverslip cultures were incubated for 48 h at 33 °C (>90% epithelial-like cells at 48 h) before treatment with 0.5 mM or 1 mM dibutyl cyclic AMP. Thereafter, replicate samples were fixed at intervals in 0.1% (v/v) glutaraldehyde and examined by light microscopy (Table 1). At 6 h, cells treated with db cyclic AMP (Fig. 2c) could not be distinguished from corresponding controls maintained at 39 °C. Cells had reverted to an elongate, spindle shape and showed no further sign of surface "blebbing". In spite of this

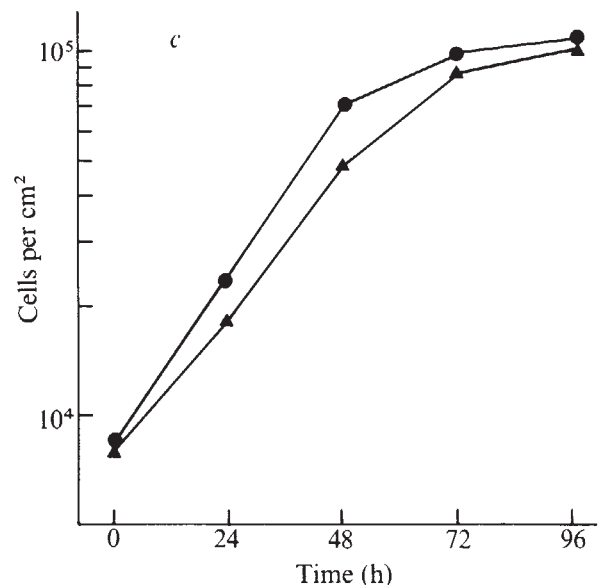


dramatic change in shape there was no effect of db cyclic AMP (0.05–1 mM for 24–72 h) on growth and cells remained quiescent at 33 °C.

Recent studies by time-lapse cinematography (our work with J. B. Clarke, in preparation) indicate that change in shape is dependent on the cell cycle: at 33 °C cells maintained their fibroblast appearance throughout the intermitotic period, rounded up for mitosis and divided but then failed to spread out on the substratum. They remained in this rounded shape indefinitely but were still firmly attached to the culture flask. Conversely, when cells were returned to 39 °C, normal morphology was only observed after mitosis. This delay of one cell cycle may be of interest from a development aspect.

A similar change of shape in the reverse direction, from epithelial to fibroblast, has been described by Puck *et al.*^{11–13} for a variant of wild-type CHO cells, clone K1, after treatment with db cyclic AMP or certain prostaglandins. An interesting comparison can be made between their published illustrations and those presented here: wild-type CHO-K1 has an identical shape to the mutant at the non-permissive temperature (Fig. 2b) while dibutyl cyclic AMP-treated cultures of CHO-K1 resemble cs4-D3 at 39 °C (Fig. 2a). Puck *et al.* have introduced the term "reverse transformation"¹³ to describe the sum of effects induced by db cyclic AMP: change in shape, ordered arrangement of confluent cultures, decreased agglutination with plant lectins, increased synthesis of collagen—the changes mirrored those found in neoplastic transformation. Cell morphology, however, was unrelated to growth behaviour and the

Fig. 1 a, Growth of cs4-D3 at different temperatures. Cells ($0.5-1 \times 10^4$; 2 ml) were seeded in screw-top glass vials (surface area, 4.3 cm²) and incubated for 48 h at 39 °C for exponential growth. Cells were then cultured further at 39 °C (■), 34 °C $\pm$ 0.1 °C (●) or 33 °C $\pm$ 0.1 °C (▲). At the times indicated, samples were trypsinised and cell numbers were estimated in a Coulter counter, model 2B1; values represent the mean for five determinations. **b**, Percentage of nuclei labelled with ³H-thymidine at 39 °C (●) or 33 °C (▲). Coverslip cultures of cs4-D3, in the exponential phase of growth, were incubated at either the permissive 39 °C or the non-permissive 33 °C. At the times indicated, triplicate samples were exposed to 6-³H-thymidine (0.1 μ Ci ml⁻¹; 5 Ci mmol⁻¹) for 30 min, washed in EBD medium, and fixed for 10 min in acetic acid-ethanol (1:3, v/v). Air-dried coverslips were mounted on microscope slides and autoradiographs were prepared using Ilford K5 nuclear emulsion. **c**, Growth of wild-type CHO cells, determined as in (a) at 33 °C (▲) and 39 °C (●).



generation time in either epithelial or fibroblast form was similar. Clone cs4-D3 illustrates a further distinction between cell shape and cell growth by resembling a normal fibroblast at 39 °C and a virally transformed cell at 33 °C.

The effects of db cyclic AMP on cell shape are antagonised by Colcemid and vinblastine, thereby implicating microtubules; this has received some support from ultrastructural studies¹⁴. We have no evidence for the involvement of microtubules in the cold-labile defect, and preliminary analysis by electron microscopy showed no significant difference in microtubule content of cs4-D3 between 33 and 39 °C.

To determine whether the mutant was defective in a specific phase of the division cycle, "labelled-mitoses

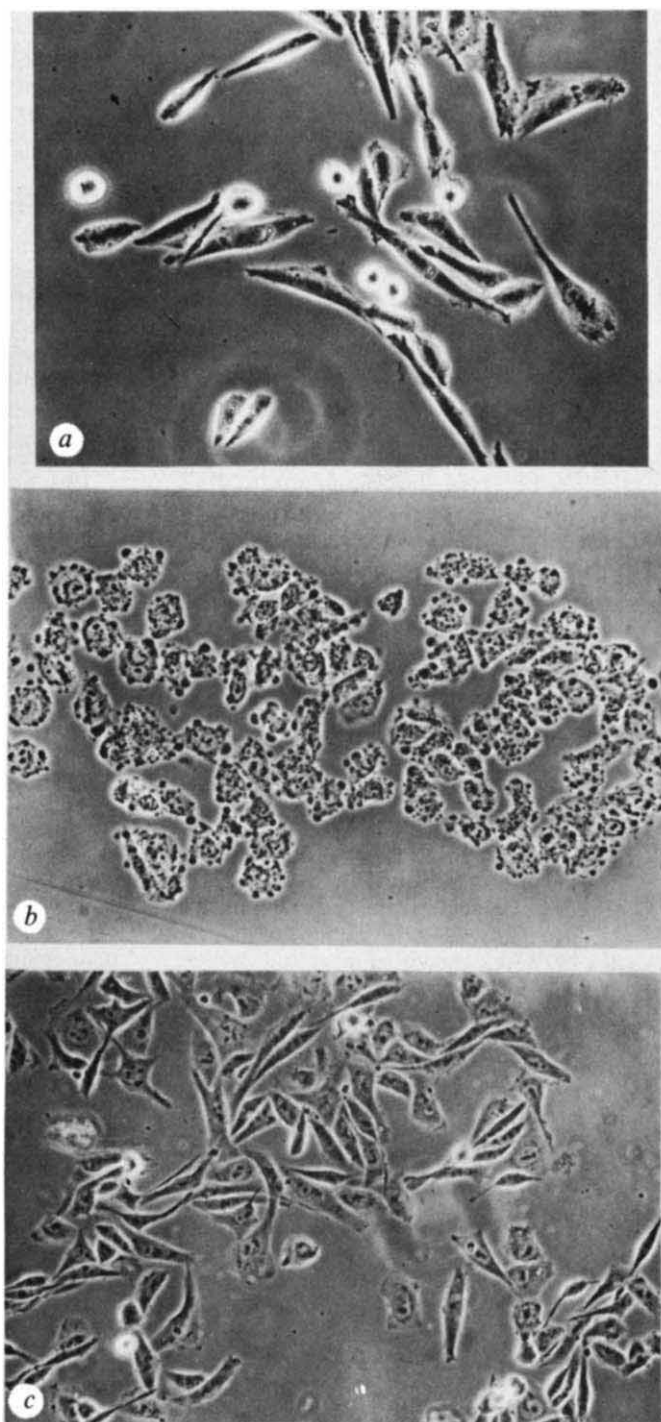
Table 1 Effect of db cyclic AMP on cell shape

Time (h)	0.5 mM db cyclic AMP		1.0 mM db cyclic AMP	
	Epithelial (%)	Fibroblast (%)	Epithelial (%)	Fibroblast (%)
0	93	7	93	7
2	70	30	26	74
4	66	34	20	80
8	35	65	16	84

Coverslip cultures which had been maintained at the non-permissive 33 °C for 48 h were treated with 0.5 mM or 1.0 mM db cyclic AMP. Coverslips were removed at intervals, washed, fixed in 0.1% glutaraldehyde and examined under light microscopy. A minimum of 200 cells was scored for morphology.

curves" were estimated at 39 °C and 33 °C. In this procedure¹³, replicate cultures are pulse labelled with ³H-thymidine and the subsequent rate of accumulation of ³H-labelled mitoses is measured, thereby providing an estimate of cell cycle traverse from S to G₂ to M. In spite of an initial low rate at 33 °C, all cells labelled with ³H-thymidine reached mitosis by 10 h (Table 2). These results together with the data in Fig. 1b indicate that cs4-D3 is arrested at the G₁ interval. Furthermore, analysis of DNA distribution profiles by flow microfluorometry in a fluorescence activated cell sorter (D. B. T. and D. F. Capellaro, unpublished results) have shown that after 24 h at 33 °C most cells

Fig. 2 Morphology of cs4-D3 at the permissive temperature, 39 °C (a); at the non-permissive temperature, 33 °C for 48 h (b), and at 33 °C for 48 h followed by a 6-h treatment with 1 mM db cyclic AMP (c) (see Table 1).



(>75%) had the 2c DNA content characteristic of G₁.

So far no mammalian *cdc* mutant has been synchronised after a temperature block and, in most instances, mutants show loss of viability by 24 h at the non-permissive temperature. In contrast, the cold-labile defect of clone cs4-D3 was reversible at all times tested and cells could be synchronised by a temperature shift (Fig. 3). Cell cultures (100–300 replicate culture vials) in the exponential phase of growth were stepped down to 33 °C for 24 h (Fig. 3a) or 48 h (Fig. 3b) or 96 h (Fig. 3c) or 240 h (Fig. 3d) and then returned to 39 °C; samples were monitored at different times for cell number and incorporation of ³H-thymidine into DNA. There was a constant and reproducible lag period followed by a three- to fivefold increase in ³H-thymidine incorporation and a plateau increase in cell number by 30 h (Fig. 3a–c). Optimum synchrony was obtained after 48 h at 33 °C (or 72 h, data not shown) and, at the first division, increase in cell number was often equal to a theoretical doubling (division index, 0.8–1.0) with respectable synchrony for at least two cell cycles. The length of the prereplicative lag before the onset of DNA synthesis was related to time spent at the non-permissive temperature. After 24 h (Fig. 3a) or 48 h (Fig. 3b) at 33 °C an 8-h lag preceded the onset of DNA synthesis; this was extended to 12 h in cultures maintained at 33 °C for 240 h (Fig. 3d). It should be emphasised that an extended delay of 12 h does not indicate loss of viability or growth potential; autoradiography studies showed that most cells could

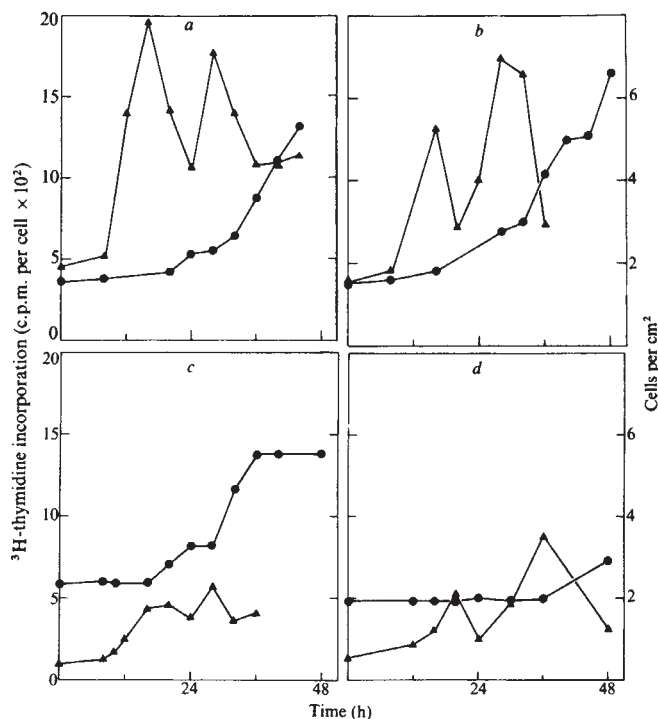


Fig. 3 Synchronous growth, after a shift from 33 to 39 °C, of cell cultures maintained for 24 h (a); 48 h (b); 96 h (c); and 240 h (d) at the non-permissive 33 °C. Each experimental group represents a minimum of 100 cultures established in glass scintillation vials. At various times, quintuplicate samples were exposed to 6-³H-thymidine (1 μCi ml⁻¹; 5 Ci mmol⁻¹) for 30 min, washed with EBD medium containing 2 mM thymidine and then ice-cold trichloroacetic acid (10% w/v) and counted directly in a Beckman liquid scintillation counter. Values for cell number represent a mean for three estimations. Cell number (●), ³H-thymidine incorporation into DNA (▲).

Table 2 Estimation of ^3H -thymidine-labelled mitoses

Time (h)	Labelled mitoses (%)	
	39 °C	33 °C
0	0	0
2	49	10
3	79	55
4	95	98
5	99	98
6	100	100

Coverslip cultures were established in Leighton tubes (10^4 cells; 2 ml), incubated at 39 °C for 48 h to ensure exponential growth, and then pulse labelled with $6\text{-}^3\text{H}$ -thymidine ($0.2\text{ }\mu\text{Ci ml}^{-1}$; 5 Ci mmol^{-1}) for 30 min. Cells were washed twice with EBD medium and re-cultured at either 39 or 33 °C. Coverslips were removed at intervals, then fixed in acetic acid-ethanol (1:3; v/v) and autoradiographs were prepared using Ilford nuclear emulsion.

be induced to divide ($> 90\%$ at 96 h) after 240 h at 33 °C.

A similar kinetic response has been reported by Augenlicht and Baserga for serum stimulation of quiescent human fibroblasts¹⁶. The length of the prereplicative phase varied according to the duration of the quiescent period. Initially, a prereplicative period of 8 h was observed before DNA synthesis and this extended to 14 h if cultures were deprived of serum for a further 4 d. Augenlicht and Baserga argued that "cells initially arrest in G_1 , subsequently pass into G_0 , and continue to go deeper into G_0 , as they remain quiescent".

cs4-D3 is remarkable in that, at 33 °C, it expresses the phenotype of normal, differentiated cells—a viable quiescent state. *In vivo* many tissues exist for an indefinite time in a quiescent, G_0 state^{17–19}, and can be induced to divide by the appropriate stimulus; for example, interaction with antigen for lymphocytes²⁰ or partial hepatectomy for liver cells²¹. It is doubtful whether the equivalent G_0 state occurs *in vitro*^{22,23}. Proliferation of normal fibroblast cultures declines at confluency and cells accumulate at the G_1 (post-mitotic) interval of the cell cycle; they can be induced to

divide further either by reculture or addition of fresh serum, but stationary cultures lose cloning efficiency after some days. Even this limited period of quiescence is absent on transformation; tumour cells continue to proliferate at low serum concentrations, albeit at a reduced rate, "piling-up" at confluency and eventually detaching from the substratum. This is particularly true for wild-type CHO: cells round up at confluency and detach from the substratum while stationary cultures, induced by isoleucine starvation²⁴, show rapid loss of cloning efficiency. It might even be argued that G_0 is a feature of the differentiated state, *in vivo*.

We suggest that cs4-D3 reverts at the non-permissive temperature from a neoplastic phenotype of the parental CHO cell to that of a "normal" cell. Phenotypic differences between normal and transformed cells have been described but many were found to be a function of cell division. An important difference between normal and transformed cells might be ability of the former to maintain a viable, quiescent state.

Received January 8; accepted April 6, 1976.

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letters to nature

Search for X-ray emission from Nova Cygni 1975

We have used the SAS-3 X-ray observatory to search for X rays from Nova Cygni 1975 before, during and after the time of optical maximum (1975 August 30–31). No X rays were detected at any time over the spectral range 0.1–50 keV, and from these negative results we can place very low upper limits on the ratio of X-ray to optical luminosity.

The light curve and spectrum of Nova Cygni 1975 show it to be a fast common nova of spectral type around A2 near maximum. (All optical and infrared data are taken from ref. 1.) Figure 1a shows the optical growth and decay of the nova, and Fig. 1b the 3σ upper limits we obtained for the X-ray intensity (1.5–15 keV) during three periods spanning the optical maximum. The X-ray measurements were taken with argon-filled proportional counters behind large angle slat collimators centred on the satellite y axis. During the observations, the spacecraft was being manoeuvred to bring Nova Cygni 1975 closer to the equatorial plane of the satellite, gradually increasing the effective observing area to the maximum of 154 cm^2 and thus lowering the successive upper limits. The upper limits were computed assuming a flat X-ray spectrum.

Two photographic magnitudes², $m_{pv} > 9.6$ on August 28.059 and $m_{pv} = 7.5$ on August 29.052, show that the nova was already brightening rapidly at the time of our first observations, August 28.5–28.9. At the time of the second observations, August 29.9–30.5, the visual magnitude was $m_v = 2.0$, implying an intensity of $5.95 \times 10^{-21}\text{ erg cm}^{-2}\text{ s}^{-1}\text{ Hz}^{-1}$ at $5,500\text{ Å}$, which gives $[I_x(\sim 4\text{ keV})]/I_{op}(5,500\text{ Å}) < 2.8 \times 10^{-8}$. We have converted these intensity ratios to luminosity ratios. With little interstellar reddening^{3,4} an A2 star has a bolometric correction of -0.3 (ref. 5) giving $m_{bol} = 1.7$ for August 29.9–30.5 and, thus, $L_{op} \simeq L_{bol} = 5.2 \times 10^{-6}\text{ erg cm}^{-2}\text{ s}^{-1}$. This yields $[L_x(1.5\text{--}15\text{ keV})]/L_{op} < 1.0 \times 10^{-4}$. Bolometric corrections derived for normal stars may, however, not be applicable to nova shells of similar temperatures. This introduces an uncertainty factor of at least 2 into the optical luminosity.

On September 1.64, repeated scans were made back and forth over the object for several minutes, giving a large increase in sensitivity. In addition, Nova Cygni 1975 was now in the circular fields of view of the thin-window, low energy (LE) proportional counters (20 cm^2 , 2.9° FWHM) and the xenon-filled proportional counters (101 cm^2 , 1.7° FWHM) so that upper limits are available for energies from 0.1 to 50 keV. These are shown in Fig. 2.

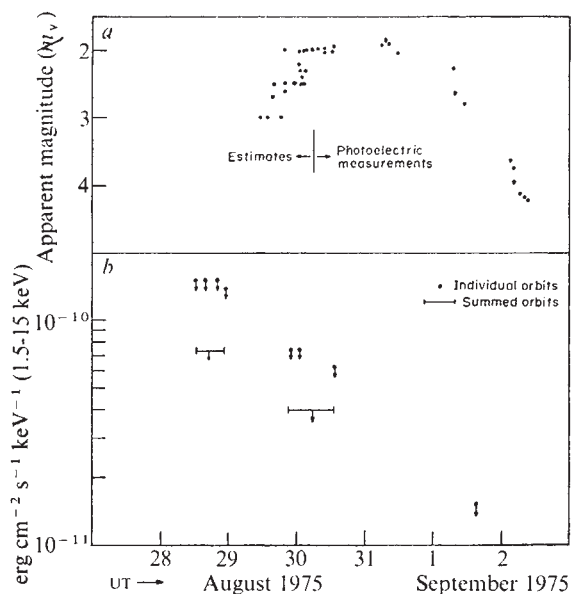


Fig. 1 *a*, Optical light curve for Nova Cygni 1975. *b*, 3σ upper limits on 1.5–15 keV X rays from Nova Cygni 1975 observed by SAS-3 at various times before, during and after optical maximum.

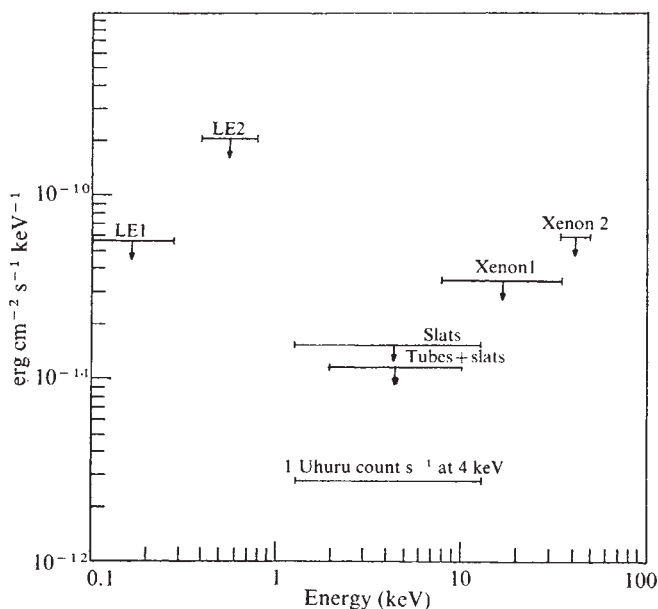
The optical luminosity decreased to $m_v = 3.1$ between our second and third set of observations, when we find $[L_x(1.5-15 \text{ keV})]/L_{op} < 8.1 \times 10^{-8}$. In the energy range 0.1–0.3 keV interstellar absorption is important. From the rapid decline after maximum, de Vaucouleurs⁶ estimated a distance of ~ 1.6 kpc. At this distance, even Sco X-1 is essentially invisible at 0.1 keV. Our upper limit of the X-ray energy received at the Earth is

$$[L_x(0.1-0.3 \text{ keV})]/L_{op} < 5.4 \times 10^{-8}$$

but this ratio may be higher at the source.

Porton and Clark⁷ reported an upper limit of 10 mJy to radio emission at 10.6 GHz near optical maximum. Hjellming⁸ reported an upper limit of 4 mJy at 8,085 MHz on September 3, soon after maximum. Three weeks later, at the same frequency,

Fig. 2 3σ upper limits to X-ray emission from Nova Cygni 1975 over the spectral range 0.1–50 keV (September 1.64). For comparison, we show the flux level corresponding to 1 Uhuru count per second, adjusted to a 1.5–15-keV energy range.



he detected weak emission (6 ± 2 mJy), which had increased to 14 mJy by October 9.

We continued SAS-3 observations of Nova Cygni 1975 after the optical maximum. The next time that Nova Cygni 1975 was close to the equatorial plane of the satellite was September 20–24, three weeks after optical maximum. No X rays were seen with the following 3σ upper limits (1.5–15 keV)

$$\text{September 20.46–20.58, } 4.4 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

$$\text{September 22.92, } 5.4 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

$$\text{September 24.9, } 4.4 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

The intrinsic properties of Nova Cygni 1975 such as peak luminosity, ejection velocity and mass are typical for common novae (see ref. 3). Because of its high relative optical brightness, we have been able to place a strong upper limit on its X-ray to optical luminosity, $L_x(1.5-15 \text{ keV})/L_o < 10^{-4}$. For comparison with known X-ray sources, we note that L_x/L_o ranges from 10^{-8} in the quiescent Sun to 10^3 in Sco X-1. Nova Cygni 1975 is the first common optical nova for which a significant X-ray upper limit has been set. What is the significance of this result?

Common optical nova outbursts are thought to result from the explosive burning and ejection of material from a white dwarf which has accreted matter over an extended period from its binary companion star^{9,10}. Since the explosion itself is caused by nuclear reactions at the base of a surface layer of material accreted on to the white dwarf, there is no *a priori* reason to believe that significant X-ray emission should directly accompany the explosive event. The spectral types of Nova Cygni 1975 and other common novae near maximum light¹¹ indicate effective temperatures of $\sim 10^4$ K. Such temperatures are entirely consistent with the small upper limit on L_x/L_o that we have obtained.

Brecher and Morrison¹², however, suggested that the subsequent shock heating of any present circumstellar material by the ejected nova shell would give rise to X-ray emission with a flux which depends mainly on the density of the surrounding gas. Wallerstein¹³ has also proposed that such a model could account in a natural way for the existence of coronal emission lines seen in novae (which in any case imply the presence of material heated to several million K, a temperature much higher than the colour temperature and absorption line temperature of the nova shell itself). In this picture, only high mass exchange systems (for example, recurrent novae such as WZ Sge, RS Oph, T CrB) should produce X-ray emission at presently detectable levels. If Nova Cygni 1975 is a virgin nova, exploding for the first time (L Jacchia, private communication), its low mass exchange rate ($dM/dt < 10^{-9} M_{\odot} \text{ yr}^{-1}$) would imply that any associated X-ray emission would be undetectable by present techniques.

This work has been supported in part by NASA.

J. A. HOFFMAN
W. H. G. LEWIN
K. BRECHER
J. BUFF
G. W. CLARK
P. C. JOSS
T. MATILSKY

Department of Physics
and Center for Space Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received January 16; accepted March 30, 1976.

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Mass loss in early stages of stellar evolution

It has been known for some time that stars lose mass between their birth on the main sequence and their death as white dwarfs (or as neutron stars or black holes for more massive stars), and the existence of the solar wind shows that not all the mass loss occurs in a last gasp to form a planetary nebula. Mass loss has also been observed in O-B stars (see refs 1-3), and in red giants⁴, but the mass loss rates observed in these stages do not seem sufficient to explain all of the mass loss required for stars to become white dwarfs. The question has always been at what stages and in what manner this excess mass was lost. Suggestions have tended to favour helium flashes for stars with $M < 2.25M_{\odot}$ and possibly double shell instability flashes for stars in the range $2.25M_{\odot} < M < 8M_{\odot}$. Although it is possible that a significant mass is lost during these stages, there is no quantitative support for this speculation, and in particular none for the helium flash. We show here that there is increasing evidence for substantial mass loss during the immediate post-main sequence stages when the star is moving from the main sequence to the giant branch on the Hertzsprung-Russell diagram.

Mass loss at these early stages of evolution may have implications on the subsequent evolution of the star and the amount of nuclearly processed material that is ejected into the interstellar medium. Although possible loopholes can be found in all of the arguments we present, it is interesting to note that the naive interpretation of each of these independent arguments points to the same conclusion.

Mäkel *et al.*⁵ have estimated the mass of Arcturus to be in the range $0.1M_{\odot} < M < 0.6M_{\odot}$. Arcturus belongs to a moving group with an age comparable to the oldest galactic clusters⁶, and to have evolved to a red giant; its original mass was $1-2M_{\odot}$. Thus it seems that Arcturus has lost a significant amount of mass.

The well known post-main sequence relation between luminosity and mass of the core (hydrogen-depleted interior) can be used to help determine the evolutionary stage of Arcturus. Calculations by Kozłowski and Paczyński⁷ show that for a star to have the luminosity of Arcturus, $\log(L/L_{\odot}) = 2.36$, the mass must be $\geq 0.3M_{\odot}$ to be moving along the giant branch, and $\geq 0.9M_{\odot}$ to be burning its helium core. Similar calculations by Paczyński⁸ indicate that a core mass of $\sim 0.52M_{\odot}$ is necessary to give this luminosity if the star is on the asymptotic branch. The luminosity is too low for Arcturus to have begun double shell flashes, and the normal composition, except for the CNO isotopes, indicates that there has been no mixing of the products of helium burning with surface layers as one may expect during double shell flashes. If the mass determined by Mäkel *et al.* is accurate, it seems most likely that Arcturus is on the giant branch and has not yet reached a helium flash point.

The composition may give additional clues as to when Arcturus lost its mass. The $^{12}\text{C}/^{13}\text{C}$ ratio has been shown⁹ to be 7.2 ± 1.5 . If the $^{12}\text{C}/^{13}\text{C}$ ratio was initially solar (90), one would expect that simple deep convective mixing while on the giant branch reduces the $^{12}\text{C}/^{13}\text{C}$ ratio from 90 to 25. Varying the initial $^{12}\text{C}/^{13}\text{C}$ ratio to 40 changes the final ratio to 20. No further change in the $^{12}\text{C}/^{13}\text{C}$ ratio is expected until the star begins double shell flashes, after which the $^{12}\text{C}/^{13}\text{C}$ ratio rapidly goes to 3.5 (ref. 10). If Arcturus has not yet reached the

double flash stage, it is difficult to understand its low $^{12}\text{C}/^{13}\text{C}$ ratio. Dearborn *et al.*¹¹ have shown that the $^{12}\text{C}/^{13}\text{C}$ ratio can be reduced below 10 if half the mass of the star was ejected before material reaching the surface convection zone had been CNO processed. (Even if Mäkel *et al.*'s mass estimate should prove low, it is possible that Arcturus started near $2M_{\odot}$ and now has a mass of $1M_{\odot}$.)

The low $^{12}\text{C}/^{13}\text{C}$ ratios observed in most K and M giants¹²⁻¹⁵ are consistent with these stars losing 20-30% of their mass before the convection zone reaches the ^{13}C -rich layer in the star. Some giants have $^{12}\text{C}/^{13}\text{C}$ ratios in the range 20-30, but are still consistent with some mass loss (10%) at an early stage of post-main sequence evolution. Arcturus cannot be dismissed as a pathological case. Dziembowski and Kozłowski¹⁶ have shown that AI Velorum stars are theoretically consistent only with hydrogen shell burning stars of masses $0.2-0.25M_{\odot}$. Stars of such low mass will never ignite helium. Kinematic studies of these stars imply progenitors with masses $\sim 1M_{\odot}$. Also the masses of the helium core of stars reaching the giant branch would be larger than those of the AI Velorum stars for $M > 2M_{\odot}$. Since the AI Velorum stars were not able to ignite helium, they must have lost mass immediately after depleting the hydrogen in their cores.

It has been known for many years that to form a white dwarf from a star more massive than $1.4M_{\odot}$, substantial mass must be lost. From the number of white dwarfs in the Hyades and from supernovae statistics, it seems that stars up to at least 3 or $4M_{\odot}$ lose sufficient mass to become white dwarfs. A white dwarf of $\sim 0.7M_{\odot}$ has been observed in the Pleiades (see ref. 17) which have a main sequence turnoff mass of $\sim 6M_{\odot}$. Integration of Reimers' formula⁴ for mass loss from a $7M_{\odot}$ star implies a mass loss of only $1.6M_{\odot}$ before carbon detonation (most of this is lost at extremely late stages). Estimates of mass ejected in planetary nebulae¹⁸ do not give sufficient leeway to make up this difference, and a $7M_{\odot}$ star must lose another $5M_{\odot}$ somewhere along its evolutionary track to form a $0.7M_{\odot}$ white dwarf. The lack of success of Reimers' formula in yielding a sufficiently large mass loss does not mean that it does not apply at all to luminous red giants, but simply indicates the need to search for an additional mechanism for losing mass.

If stars lose 20-30% (or even 50%) of their mass when subgiants, there are few directly observable consequences. The luminosity and temperature of most field stars are affected more by unknowns such as age and original mass than mass loss. One would expect, however, to see some effect in the Hertzsprung-Russell diagram of clusters if mass is being lost. First, as was shown by Chiosi and Naisi¹⁹, mass loss on or near the end of the main sequence causes a slightly smaller core to form, and stars approach the giant branch with a lower luminosity. Second, if different amounts of mass are lost from stars in the same clusters, there will be a spread in the horizontal branch or clump (for Pop. I stars with $M < 3M_{\odot}$). Rood²⁰ has shown that Hertzsprung-Russell diagrams of globular clusters are consistent with a mass loss of $25 \pm 3\%$. Varying masses on the giant branch also seem consistent with Osborn's observations of the old open clusters, M67 and NGC2420 (refs 21, 22), and both of these effects also appear in the observations of Flower²³; he observed several young clusters with $7M_{\odot}$ turnoff masses where there seem to be giant stars of mass $5M_{\odot}$. Lindhoff^{24,25} reported on such effects in several galactic clusters, but uncertainty in the turnoff mass (or age, ref. 26), or small numbers of stars made interpretation difficult.

Table 1 is a chart which summarises all of the arguments presented and strongly indicates the possibility that mass loss occurs as a subgiant or at the base of the giant branch (before the convection zone reaching the ^{13}C layer).

One should remember that none of the above arguments is conclusive. Other explanations may be found for the scatter in young clusters, $^{12}\text{C}/^{13}\text{C}$ ratios, and the apparent low mass of AI Velorum stars. The arguments stated here may be just a large collection of false leads.

Table 1 Summary of evidence for subgiant mass loss.

	Main sequence	Subgiant	Giant branch	Helium flash	Horizontal branch	Asymptotic branch	Double shell flashes	Supernovae	Planetary nebula	Total fractions of main sequence mass which is lost
O-B Supergiants	*								X	?
Luminous red giants						*	*		*	10%
Globular clusters	X				X	X	X	X		20–30%
White dwarf in Pleiades				X				X		90%
Arcturus	X	*	*				X	X	X	> 50%
$^{12}\text{C}/^{13}\text{C}$ several K giants	X	*	*			X	X	X	X	20–30%
AI Velorum stars	X			X	X	X	X	X	X	≈ 70%
NGC 2420	X				X	X	X	X	X	≈ 30%
Scatter on H-R-diagrams	X			X	X	X	X	X	X	?

*Requires mass loss in this stage.

X, either this stage did not occur, or only small amount of mass loss allowed.

Blanks indicate possibility of mass loss at that stage but no strong evidence.

Mass loss at an early stage can reduce the number of carbon detonation supernovae (which seem to produce excessive amounts of iron peak elements) and allow stars to arrive at the double shell flash stage with smaller envelopes, making it easier to produce carbon stars and FG Sag like objects. If, however, stars lose mass as subgiants or low luminosity giants, it is necessary to ask why some low mass stars, such as those in globular clusters, lose only 25% of their mass so that they can still undergo a helium flash, whereas AI Vel stars lose too much mass to even ignite helium (if Dziembowski and Kozłowski's models are correct). This question is, of course, entangled in how the mass is lost.

It is possible to estimate the energy constraints on the mass loss. The power per unit volume in a convection zone from acoustic²⁷ and gravity²⁸ waves are

$$P_{\text{acc}} = 38 \frac{\rho V_c^8}{l V_s^5}$$

$$P_{\text{grav}} = 100 \frac{\rho V_c^3}{l} \left(\frac{l}{H} \right)^5$$

where ρ is the density, V_c is the convective velocity, V_s the sound velocity, l the mixing length, and H the pressure scale height. Unfortunately, both powers end up being proportional to l^7 —an extremely uncertain parameter.

The acoustic energy flux is sufficient to sustain a tremendous corona and could easily provide the required mass loss to produce the $^{12}\text{C}/^{13}\text{C}$ ratios. If, however, this is the power source of the solar corona, then only a small fraction of the flux emerges to drive the solar wind. Calculations of the emergent flux have been attempted by Whitaker²⁹ and DeLoore³⁰, but are extremely uncertain. There is also no theory explaining the possible effects of metal concentrations, magnetic field or rotation to explain the apparent variation of the mass loss.

Those arguments strongly suggest the possibility of substantial mass loss at an early stage of post-main sequence stellar evolution. Further observations are required for some indication of mass loss, such as of coronae, particularly of stars which have recently left the main sequence and are in the process of moving to the giant branch. They also indicate the need to look in detail for a reasonable mechanism to explain the required mass loss.

We would like to thank B. Paczynski for numerous discussions. This research was supported in part by a Smithsonian Foreign Currency grant and by the NSF.

D. S. P. DEARBORN

*Institute of Astronomy,
Warsaw, Poland and
Institute of Astronomy,
Cambridge, UK*

M. KOZŁOWSKI

*Warsaw University,
Warsaw, Poland*

D. SCHRAMM

*University of Chicago,
Chicago, Illinois 60637*

Received January 21; accepted April 1, 1976.

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Accretion-disk scenarios of the precursor peak in X-ray transients

ONE of the striking features of a number of the transient X-ray sources is the 'precursor peak', an intensity maximum which precedes the rise to primary maximum and is separated from it by a dip. It is characteristic of A0620–00 (refs 1, 2), A1524–62

(ref. 3), A1118-61 (refs 4, 5), and A0535+26 (refs 6-9). The time scale across the precursor and subsequent dip is $\sim 4-20$ d, though for A0620-00 the dip itself spans a time of only ~ 10 h. Apart from flares, which have a much shorter time scale, there are similar secondary peaks observed during decay for some of the sources—A1524-62 and A0535+26, for instance. Any model of these transients must account for such features along with the concurrent softening of the X-ray spectrum in some cases, the temporal asymmetry of the light curve and, for sources A1118-61 (ref. 5) and A0535+26 (ref. 6), periodicities of 6.755 ± 0.010 min and 104.14 ± 0.16 s, respectively. It is possible to explain qualitatively the precursor and related phenomena on the basis of accretion-disk behaviour with a rising accretion rate $\dot{M}$, followed by its gradual subsidence and the emptying of the disk. In this paper I present three schemes.

I start with two preliminary comments. There are models where a decreasing $\dot{M}$ gradually uncovers the X-ray producing regions buried beneath an optically thick blanket of accreting material¹⁰. This seems unlikely for a number of reasons (A. P. Willmore, unpublished)—the gradual softening of the spectrum and the skewness of the light curve towards the decay phase.

Second, our reliance on available thin-disk models is, at this stage, not crucial for our work. Except for those of Lightman^{11,12}, all models are either independent of time^{13,14} or time averaged¹⁵, assuming that $\dot{M} = 4\pi r h \Sigma v_r$ is constant; here r is the radius, h the disk half-thickness, $\Sigma = \Sigma(r)$ the surface density and v_r the radial drift velocity. Nevertheless, their use in the present context is rigorously justified as long as the time scale t_M over which $\dot{M}$ varies satisfies¹².

$$t_M > t_D \quad (1)$$

where $t_D \simeq (r/h)^2 \alpha^{-1} t_H$ is the drift time scale, the time it takes Σ to diffuse a distance r . Here α is the 'catch-all' viscosity parameter and t_H is the hydrodynamic time scale; $\alpha \simeq 10^{-3}-1$, $t_H \simeq 10^{-3}-10^{-2}$ s. Thus, if $\dot{M}$ is increasing over a period of days, equation (1) will generally be satisfied. Even when it fails, the models probably give us a qualitatively correct picture of the evolution.

In the first scenario, Roche-lobe overflow begins and increases; a disk forms. At first, the very soft X-ray flux from portions of the disk with large radii will dominate. But eventually, as a stable type II (electron-scattering opacity and matter dominated) inner region develops, the luminosity will become significant in the 1-20-keV range, where the Arie V transients have been detected. The continuing rise in $\dot{M}$ will lead to increasing luminosity, since the flux $F \sim \dot{M}$ throughout the disk^{13,14}.

For the two sources A1524-62 (ref. 3) and A0620-00 (ref. 2) a significant spectral softening is evident during the luminosity increase, but at different phases of the light curve. In the former it occurs during the rise to the precursor peak; for A0620-00, however, the spectrum remains relatively hard across the precursor, with the softening occurring only afterwards—during the rise to principal maximum. How is this to be interpreted?

The intensity dip is caused, in this scheme, by disk thickening, primarily in the cooler outer regions, which absorb some of the radiation originally destined for the observer. Some of the harder photons from the inner regions of the disk and/or from the surface of the compact object, are reprocessed and re-emitted at lower energies (Cunningham, C. T., unpublished). This mechanism can account for the spectral softening, which in A1524-62 is in proper correspondence with the intensity dip for this picture: thickening (and spectral softening) occurs during the precursor peaking and then reaches a point where the outer region of the disk begins to intercept radiation directed towards the observer. For A0620-00, however, this correspondence fails—and it seems impossible to apply the scenario properly.

The absence of spectral softening (as in A1118-61 (ref. 5)) does not necessarily rule out this scheme. Whether it will be

observed in a given case depends on the observer's angle of inclination and other factors and can be predicted only on the basis of a detailed quantitative model.

The gradual thickening postulated here would arise primarily from the reabsorption of radiation itself—from the hotter inner regions of the disk and from the surface of the compact object. As the disk thickens, of course, it becomes even more susceptible to such absorption and reheating. Thus it is possible that through this process the outer regions would swell into a cloud shrouding the entire compact object and inner-disk system.

The continuing increase in $\dot{M}$ forces the luminosity of the thick disk or cloud configuration from the dip to the primary maximum. Then, as $\dot{M}$ begins to decrease, the transient enters its decay phase, which has a time scale of months. It is likely that this is just the emptying time t_e of the disk modulated by $\dot{M}$. From both related observational (Bailey, J., unpublished) and theoretical¹⁶ considerations, t_e , for reasonable values of $\dot{M}/\dot{M}_\odot$, binary period and disk radius, is of the required order. If an extensive quasi-spherical cloud shrouds the disk, its accretion time may also be important. A secondary maximum during decay, such as is seen in A0535+26, can be accounted for in terms of thick disk subsidence to a thin disk.

For sources like A1118-61 and A0535+26, it is important to establish whether their periodic components persist at the same level through the peak and dip and on into the principal maximum, or are characteristic only of certain stages of the transient's evolution. Such information would also help determine whether the precursor phenomenon arises primarily from disk behaviour or from mechanisms associated with, say, funnel accretion on to the polar caps of a neutron star. Though these observed periodicities are not directly explained by the disk behaviour itself in this scheme, they are not inconsistent with it. They may be a characteristic, for example, of the component of radiation emanating from the polar caps of the neutron star in a neutron-star thick-disk system.

The second scheme pertains to non-magnetic situations, black holes or unmagnetised neutron stars, and applies primarily to A0620-00. It begins and ends like the first. But, in the course of the rise to the precursor peak with increasing $\dot{M}$, either an unstable radiation dominated inner region appears^{11,12} or thermal instabilities¹⁸ develop from the severe density thinning across the inner edge (W.R.S., unpublished). In either case the inner region of the disk will blow up into a very hot, essentially spherical cloud, and inner disk models like those of Thorne and Price¹⁹, Lightman and Shapiro²⁰, Eardley *et al.*²¹, and Shapiro *et al.*²² will be applicable. The onset of such instabilities occurs at the precursor peak, and the subsequent dip is formed as the luminosity contributed to the 1-20-keV range by the hotter inner region is lost. The continually increasing $\dot{M}$, however, quickly overcomes the dip and moves the transient towards its maximum.

This sequence would explain why the spectrum remains hard during precursor peaking in a source like A0620-00 and begins softening only afterwards. It would also account for the relatively sudden dip with recovery on a time scale of hours. A crucial test of the model would be significant spectral softening in the narrow dip itself.

It is possible that, corresponding to the occurrence of the dip, hard X-ray emission in the 100-500 keV range from the very hot inner cloud would make its appearance. But, then again, such a source might not be evident, particularly if this cloud is optically thin. For then the flux $F \sim n^2 T^4$ instead of the much more substantial $F \simeq bT^4$ of black-body radiation. The swollen inner region, however, might still function to boost soft photons into medium hard X-ray range by inverse Compton scattering^{21,22}. G. F. Carpenter, M. J. Coe, A. R. Engel and J. J. Quenby (unpublished), in fact, have shown that the spectrum of A0620-00 is similar in many respects to that of Cygnus X-1, where just such a model closely fits the data^{21,22}.

The third scenario applies only to a black hole in a stellar-wind environment and demands both an increasing $\dot{M}$ and an in-

creasing specific angular momentum L of the accreting material. Modulation of L could be provided by a highly eccentric binary orbit, but this seems somewhat questionable on other grounds²³. The general picture involves the formation at very small radii of an initial marginal accretion disk^{24,25} ($r=4M$ to $r=12M$ in the Schwarzschild case—in units where $G=c=1$), which grows into a stable disk as $\dot{M}$ and L increase.

We can describe the disk evolution in terms of the orbital topography of the Schwarzschild metric²⁶. The specific energy (including rest mass) of the infalling material is $E \approx 1$. As L approaches and then becomes larger than $L_{ms} \approx 3.464M$ (the specific angular momentum for a marginally stable circular orbit at $r_{ms} \approx 6M$), a chaotic transitional disk will form, heat up and radiate²⁵. Such a disk will extend outwards, not from r_{ms} , but from $r_{mb} \approx 4M$ (the marginally bound circular orbit). Since the material has both a large E and $L < 4M$, a large proportion of it will be in unstable circular and elliptical, and stable elliptical, orbits extending from r_{mb} outwards ($E_{mb} = 1$ and $L_{mb} = 4M$). As the material loses some of its energy and angular momentum, a large proportion of that between $4M < r < 6M$ will first migrate into stable circular orbits at $r > r_{ms}$ before spiralling on to the secondary.

As L and $\dot{M}$ increase, the disk grows radially, and the inner region between $4M$ and $6M$ disappears, superseded by a stable 'classically' described thin disk with inner edge at r_{ms} . The formation of the chaotic inner disk would explain the initially hard spectrum, the precursor peak would arise from the gradual build up of this inner disk with increasing $\dot{M}$ and L , and the dip, along with some of the spectral softening during the rise to primary maximum (as in A0620—00), to the dissipation of the transitional disk as the stable outer disk developed. The rise to primary maximum and the continuing softening of the spectrum would then be the result of both the gradual growth of the stable disk and the increasing $\dot{M}$. The time scale across the precursor peak will depend on how long accretion with a marginal L continues, and that across the dip on the time it takes for the rising accretion rate to recover the ground lost through dissipation of the chaotic disk—for A0620—00 this would have to be ~ 10 h. The time scale of decay, again, is just t_e modulated by $\dot{M}$. A marginal-disk stage should be a briefly discernible feature during the decline.

I hope this work will open theoretical avenues of approach to the precursor phenomenon and motivate further investigation of this unexpected feature.

I thank Professor Martin Rees for his help and A. C. Fabian and J. E. Pringle for discussions.

W. R. STOEGER, S. J.

*Institute of Astronomy,
Madingley Road,
Cambridge CB3 0HA, UK*

Received February 16; accepted April 7, 1976.

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A return to the pre-1971 intensity level and a 5.6-d modulation for Cyg X-1

CYX X-1 exhibited three pronounced X-ray intensity increases during 1975. The last of these, unlike the previous two, has not rapidly decayed back to its pre-increase value. Such a persistently high emission level from Cyg X-1 has not been observed since the source 'transition' in 1971¹.

A 5.6-d modulation synchronised with HDE226868 was observed over the first six months of the Ariel V all-sky monitor (ASM) data accumulation before the April 1975 increase², but was not detectable immediately afterwards. We report here the detection of a similar 5.6-d modulation in the 'high-state' data obtained over the 3-month interval November 1975–January 1976.

The Ariel V ASM experiment has monitored Cyg X-1 quasi-continuously since its launch in October 1974. No flare-like increases were observed until April 1975, when the source suddenly increased to an intensity level in excess of that of the Crab Nebula. The rise³, maximum⁴ and decay⁵ were marked by considerably more variability on time scales of hours, or more than was the relatively quiescent pre-increase temporal record. A smaller increase was reported in September⁶, which displayed the same variability and decay time of ~ 1 week. The third increase in November^{7,8} was to the level of the April event, displaying the same variability. It did not, however, decay over the same time scale⁹, and has shown no sign of decay after 3 months. Figure 1 displays the daily averages of the Cyg X-1 data accumulated between launch and January 31, 1976 by the ASM.

The folded data from both the six months before the April 1975 flare and the 3-month interval November 1975–January 1976 are displayed in Fig. 2, where we have used the ephemeris of ref. 10 to define the epoch of superior conjunction. The folds are in five bins, with the ordinate for each bin defined as the source intensity relative to the average of the four bins excluding superior conjunction. This average value for the new data is more than twice that of the pre-April 1975 measurements.

The light curve decrement (that fraction of the total source emission over one 5.6-d cycle which is deficient at superior conjunction) is 0.019 ± 0.003 for the lower trace of Fig. 2, compared with 0.029 ± 0.004 for the upper trace. This level of modulation was not detectable in the ASM data between the April and November flares, which may have been the result of uncorrelated source intensity variations masking the effect (the experimental value of the decrement was 0.008 ± 0.009 during this interval). Daily averages for the April, September and November–January increases are displayed in Fig. 3, combined with a 5.6-d grid from the ephemeris of ref. 10. It is obvious that the uncorrelated intensity variations prevent the direct observation of the reported level of 5.6-d modulation over individual cycles in general, and the effect can only be demonstrated convincingly by averaging over many cycles.

In ref. 2 it was suggested that the modulation observed before the April flare might be associated with the build up to the increase, and might be observable only just before such increases. The new results reported here suggest that this level of X-ray modulation may be generally character-

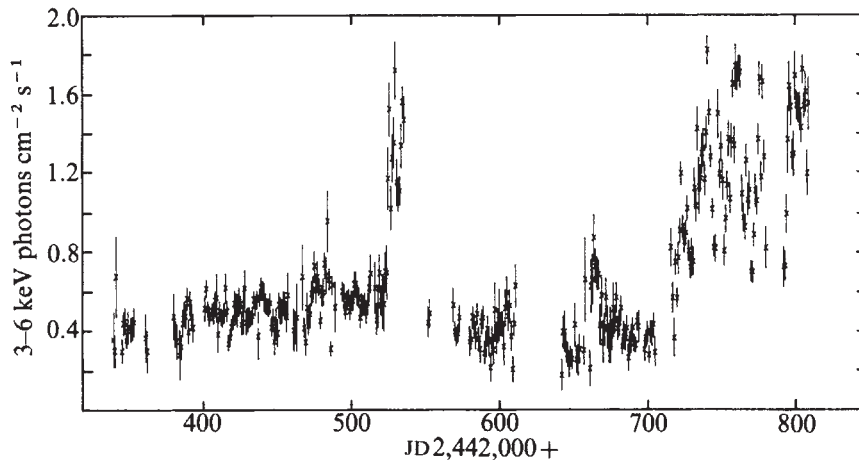


Fig. 1 Daily averages for more than one year of ASM measurements of Cyg X-1. The ordinate is natural for the experiment, with the Crab Nebula giving ~ 1.2 in the same units.

istic of Cyg X-1. The orbital analyses of Hutchings (see ref. 11) and Bolton¹⁰ suggest that an inclination i of $\sim 30^\circ$ is necessary to reconcile the optical measurements, in contrast to the $i \sim 60^\circ$ required if the present results represent an eclipse of the source by a completely-filled primary Roche lobe. It would seem, therefore, that our results are most easily explained by scattering from ionised material between the two stellar components which extends outside

the orbital plane (see ref. 10). The material must be largely ionised because a much larger effect has not been reported at lower energies. As described in ref. 9, the magnitude of the present level of modulation is approximately an order of magnitude in excess of the expectation from the more

Fig. 2 Data taken before the April 1975 increase (upper trace, November 1974–April 1975) and after the start of the November 1975 increase (lower trace; November 1975–January 1976) folded in 5 bins using the HDE226868 ephemeris¹⁰.

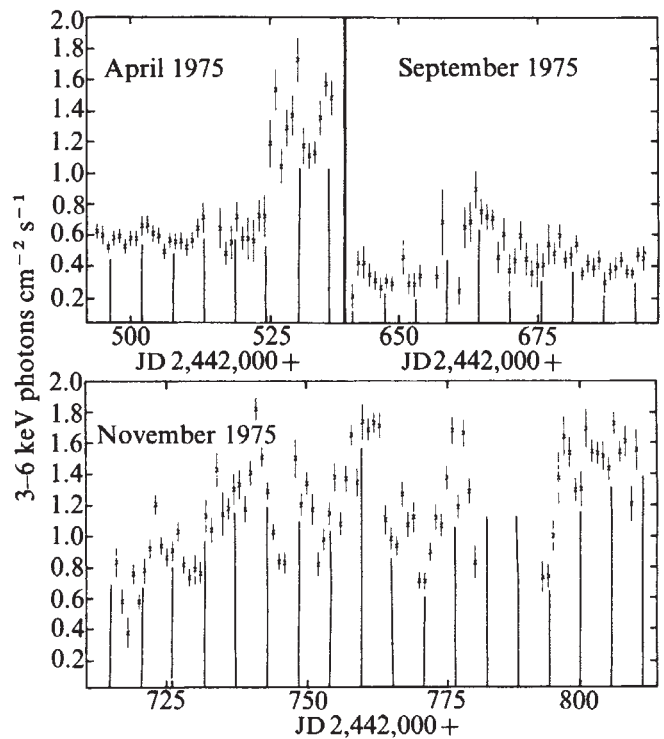
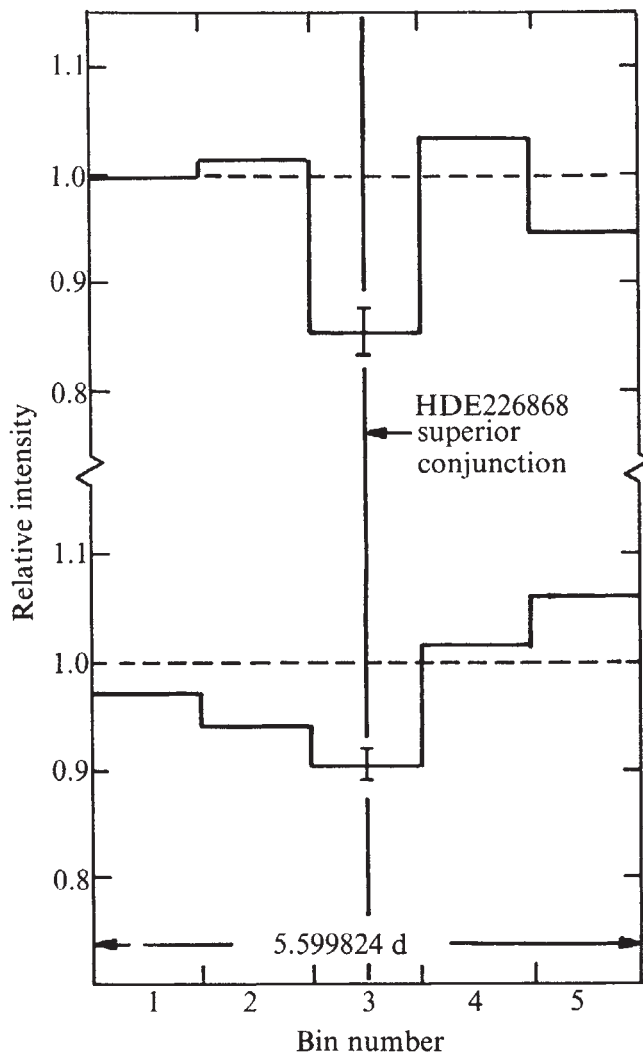


Fig. 3 Daily averages at the times of the three 1975 increases, with a superposed HDE226868 superior conjunction grid. On a finer time scale, the asynchronous intensity variations are even larger than those reflected in the plotted daily averages.

sharply defined 'absorption dips' which have been attributed to the effect of neutral material, so that it is not at all clear how these two classes of superior conjunction decrements are related. In any case, the decrement at > 3 keV reported here appears to be consistent with a stable few per cent underlying average periodicity in both the high and low intensity states of Cyg X-1. We cannot ascertain unambiguously whether this effect is present every cycle at the average level, or whether the effect is larger and present only a fraction of the time. Figure 3 does contain a few pronounced minima which are synchronised with superior conjunction, but it also contains many which are not. The final determination of this question must await a detailed analysis of many high state cycles (of which only ~ 15 are

included here). Should the intensity remain high indefinitely, the ASM should be capable of resolving the issue eventually, as it is expected to continue operation for at least another year.

L. J. K. acknowledges support from the University of Maryland.

Note added in proof: Shortly after this manuscript was submitted, Cyg X-1 was observed by the ASM to return sharply to its "low" state¹². Its average intensity for the week ending on JD 2,442,822 was 1.85 ± 0.04 , after which it dropped by a factor of three in one week. The intensity has since been roughly constant at ~ 0.5 to JD 2,442,851.

S. S. HOLT
L. J. KALUZIENSKI
E. A. BOLDT
P. J. SERLEMITSOS

Laboratory for High Energy Astrophysics,
Goddard Space Flight Center,
Greenbelt, Maryland 20771

Received February 17; accepted March 18, 1976.

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Molecular hydrogen formation on interstellar dust grains

EARLY estimates of the maximum temperature, T_M , of interstellar dust grains (IDGs) consistent with efficient H_2 formation on their surface, yielded values near 10 K (refs 1–3). The expression for T_M is

$$T_M = E_H / \ln \left[\frac{1}{S v n_H A \tau_0} \right] \simeq 10 \text{ K} \quad (1)$$

where E_H is the binding energy of hydrogen to the dust grain $\simeq 400$ K, S is the sticking coefficient of H on the grain $\simeq 0.5$, v is the average velocity of H atoms in the dust clouds $\simeq 10^6$ cm s⁻¹, n_H is the hydrogen density in the cloud $\simeq 10^3$ cm⁻³, A is the surface area of a dust grain $\simeq 10^{-9}$ cm², and $\tau_0 \simeq 6 \times 10^{-13}$ s. Observations have shown this T_M to be unacceptably low (see, for example, refs 5 and 4), and have led to proposals of new or revised mechanisms for interstellar H_2 formation^{5–9}. Here we point out that for a certain class of gas-solid systems the characteristic desorption lifetime, τ_0 , is many orders of magnitude longer than that used in refs 1–3. For grains made of these materials the original "physisorption model"^{1–3} will work for grain temperatures up to 30 K.

The τ_0 of 6×10^{-13} s, used in refs 1–3, comes from the Frenkel theory of adsorption¹⁰, in which detailed balance, single particle partition functions, and equilibrium between the gaseous and adsorbed phases are assumed. These assumptions are not necessarily valid in interstellar clouds. A dynamical theory of desorption makes none of the aforementioned assumptions. Ying and Bendow¹¹ have formulated such a theory by considering phonon-induced desorption. In particular, they performed detailed calculations for the model system, Ne adsorbed on Xe-covered graphite, and found that τ_0 exceeded 10^{-5} s for temperatures below 40 K. This occurs because the low temperature excitation spectrum of the substrate (Xe on graphite) contains few phonons with energies exceeding

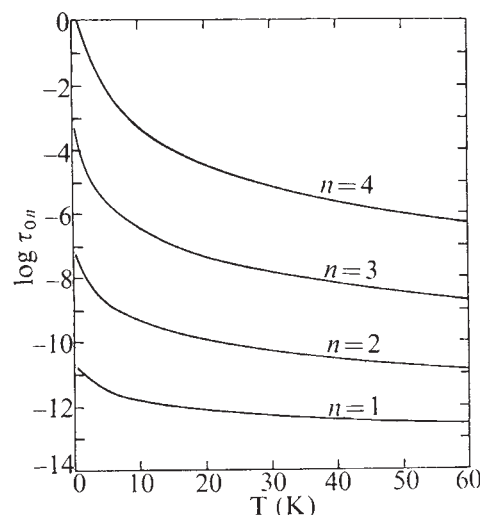


Fig. 1 $\log \tau_{0n}$ as a function of T for $n = 1, 2, 3$ and 4. τ_{0n} is related to the average lifetime with respect to desorption, τ , by the Frenkel formula¹⁰, $\tau_{0n} = \tau \exp(-E/kT)$, where E is the binding energy of the adsorbed atom and T is the temperature of the substrate.

the binding energy of the adsorbed atoms. Thus multiphonon processes, which are slow when the phonon gas is dilute, are necessary for desorption. A similar situation does not occur for H adsorbed on the graphite basal plane—a commonly proposed form of IDG. The binding energy of H on graphite, E_H , is about 400 K (ref. 12), while the transverse and longitudinal Debye temperatures are $T_t = 760$ K and $T_l = 2,700$ K (ref. 13), respectively. Desorption induced by a single phonon can thus occur and a short τ_0 is the result. This can be seen from the following derivation. The flux of phonons, each with sufficient surface normal energy to cause desorption, times the phonon-atom cross section, gives the desorption rate, R ,

$$R = \int_{\omega_0}^{kT_D/\hbar} \int_{\theta, \psi} c g(\omega) \sigma(\omega, \theta) \cos^2 \theta \sin \theta d\theta d\psi d\omega \quad (2)$$

where $\omega_0 = E/\hbar$, E is the binding energy of the adsorbed species to the substrate, T_D is the Debye temperature of the substrate (all polarizations are treated equivalently), c is the speed of sound in the substrate, $\sigma(\omega, \theta)$ is the cross section for maximum energy transfer between the phonon and the adsorbed atom $\simeq \pi(\lambda + d)^2$, $\lambda(\omega)$ is the phonon wavelength, d is the adsorbed atom scale size, $g(\omega)$ is the phonon distribution function in the substrate¹⁴

$$(g)\omega \simeq V(3/2\pi^2 c^3) \{ \omega^2 / [\exp(\hbar\omega/kT) - 1] \} \text{ s for } \frac{\hbar\omega}{k} \leq T_D$$

and
$$g(\omega) = 0 \text{ for } \frac{\hbar\omega}{k} > T_D$$

For the hydrogen-graphite system, $E = E_H \simeq 400$ K, $c \simeq 10^6$ cm s⁻¹, $T_D \simeq 1,500$ K, and $d \simeq 3 \times 10^{-8}$ cm.

Using the facts that $kT \ll E_H < kT_D$ and $\lambda_0 \lesssim d$, equation (2) can be integrated

$$R \lesssim \frac{d^2 V}{2\pi^2 c^2} \frac{kT}{\hbar} \omega_0^2 \exp(-\hbar\omega_0/kT) \quad (3)$$

This gives a desorption time of the Frenkel form and magnitude,

$$V/R \leq \tau_1 = \tau_{01} \exp(\hbar\omega_0/kT) = \tau_{01} \exp(E_H/kT) \quad (4)$$

where

$$\tau_{01} = \frac{2\pi^2 c^2}{d^2} \frac{\hbar}{kT} \frac{1}{\omega_0^2} \simeq 6.0 \times 10^{-13} \text{ s (at } T=100 \text{ K)}$$

The inequality in equations (3) and (4) comes from the un-

Table 1 Characteristic desorption times of certain adsorbed gas-substrate systems

Adsorbed species	Substrate	Temperature (K)	Binding energy (K)	τ_0 (s)	Reference
H ₂	H ₂ covered Cu	1-4	96	4×10^{-9}	23
H ₂	CO covered Cu-Ni	10-25		10^{-7}	19
He	He covered Cu-Ni	4-30	30-90	1.5×10^{-7}	21
CO ₂	SiO ₂	80-130		5×10^{-4}	20
H ₂ κ state	CO covered W	300	3,700	1.25×10^{-3}	18
CO	CO covered Co	300-1,000	2,000	2×10^{-3}	22
CO	CO covered Ni	300-1,000	2,000	4×10^{-3}	22
Ar	SiO ₂	80-130	250-500	1	20

certainty in the cross section. Specifically, $\sigma(\omega, \theta)$ depends on the phonon polarisation, the availability of final phonon and adatom states, the scattering matrix, and the constraints of energy and momentum conservation.

Graphite is an oddity in that it has a very high Debye temperature. "Softer" materials of low atomic mass constituents such as α -iron, ice, silica or solid carbon monoxide, have Debye temperatures below 200 K (refs 15 and 16). For hydrogen physisorbed on these materials ($E_H \geq 400$ K) two or more phonon impacts are necessary for desorption. For two-phonon desorption to occur, a second phonon must transfer sufficient energy to a hydrogen atom which has been raised to an excited (surface normal) vibrational state by a previous phonon. The second phonon must impact on the excited hydrogen with proper phase and within a time $t \approx 2\pi/\omega_0 \sim 1.2 \times 10^{-13}$ s, or else the excitation may decay away by spontaneous phonon emission. The time between successive impacts by energetic phonons is $\tau_1(\omega)$, equation (4). Accordingly, the probability that an excited atom will be desorbed by the impact of a second phonon is

$$P(\omega) \approx \frac{1.2 \times 10^{-13} \gamma}{[\tau_1(\omega) + 1.2 \times 10^{-13}]} \quad (5)$$

and the desorption time (at low temperatures) is

$$\tau_2 \approx \frac{\tau_1(\omega')}{P(\omega)} = \frac{1}{1.2 \times 10^{-13} \gamma} \left[\frac{2\pi^2 c^2}{d^2} \right]^2 \left[\frac{\hbar}{kT} \right]^2 \frac{1}{\omega^2} \frac{1}{\omega'^2} \exp[\hbar(\omega + \omega')/kT] \quad (6)$$

where γ is a phase coherence factor for the two phonons ≈ 0.5 (this corresponds to a $\pi/3$ phase shift between phonons, thus a 50% decrease in energy due to interference effects), $c \approx 5 \times 10^4$ cm s⁻¹ for iron, ice, silica, and so on, $\hbar\omega$ and $\hbar\omega'$ are the energies of the two phonons, and $\hbar(\omega + \omega') \geq \hbar\omega_0$, the binding energy of the adsorbed hydrogen. The minimum τ_2 occurs for

$$\omega = \omega' = \omega_0/2 \quad (7)$$

that is, the maximum rate of two phonon desorption occurs when the phonons have equal energy.

This can be generalised to n phonon processes. For n phonon-induced desorption, τ_n is given by

$$\tau_n = \left[\frac{1}{1.2 \times 10^{-13} \gamma} \right]^{n-1} \left[\frac{2\pi^2 c^2}{d^2} \right]^n \left[\frac{\hbar}{kT} \right]^n \frac{1}{(\omega_0/n)^{2n}} \exp(E_H/kT) \\ \approx (n)^{2n} \left(\frac{200}{T} \right)^{n-1} \frac{1.5 \times 10^{-11}}{T} \exp(E_H/kT) \text{ s} \quad (8)$$

which defines a characteristic desorption time

$$\tau_{0n} = (n)^{2n} \left(\frac{200}{T} \right)^{n-1} \frac{1.5 \times 10^{-11}}{T} \text{ s} \quad (9)$$

A graph of τ_{0n} as a function of temperature for $n = 1, 2, 3$ and 4 is shown in Fig. 1. It must be stressed that these are lower limits for τ_{0n} because of the uncertainty in $\sigma(\omega, \theta)$. For a four-phonon process, as would be necessary for H desorption from solid CO or to covered surfaces, τ_{04} is 10^{-5} s and T_M (equation (1)) is raised to 30 K.

Long τ_0 s have been measured in many experiments. As early as 1928, Cockcroft¹⁷ measured τ_0 of 10^{-9} s at $T = 150$ K. Later experiments showed τ_0 values up to 1 s and several notable examples are listed in Table 1. As can be seen, τ_0 for H₂ on CO covered W¹⁸, for H₂ on CO covered Ni¹⁹ and for CO₂ and Ar on SiO₂ (ref. 20) are greater than 10^{-7} s.

In summary, a dynamical theory of phonon-induced desorption is presented. It is shown that the characteristic desorption time, τ_0 , is long at low temperatures if the binding energy of the adsorbed atoms exceeds the Debye temperature of the substrate. This fact has immediate application to the problem of abundant H₂ present in interstellar dust clouds by raising the maximum temperature for efficient H₂ formation to 30 K. From this model we conclude that the basal planes of clean graphite flakes are not effective substrates of H₂ formation, whereas solid CO, H₂O or SiO₂ surfaces are.

I thank E. S. Marmar, J. L. Cecchi, and J. G. King for discussions. This work was supported by the USERDA.

S. A. COHEN

Plasma Physics Laboratory,
Princeton University,
Princeton, New Jersey 08540

Received January 27; accepted March 30, 1976.

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Isotopic fractionation in meteoritic cadmium

ISOTOPE and elemental abundance studies of Cd in this laboratory have led to the discovery of Cd with an isotopic composition which differs significantly from that normally observed in nature. This isotope abundance study was initiated with the primary aim of detecting and measuring neutron irradiation effects in Cd (¹¹³Cd (n, γ) ¹¹⁴Cd, $\sigma = 20,000$ barn) of similar origin to that observed in Gd (ref. 1), and included a survey of

Table 1 Measured isotopic ratios for cadmium in Brownfield

Date of analysis	Sample† identification	No. of data sets	Sample† size (ng)	106/112*	108/112	110/112	111/112	113/112	114/112	116/112
May 22, 1974	AML.H15.81	15	389	0.05371 (0.00010) -8.7‰	0.03800 (0.00009) -1.3‰	0.52337 (0.00049) -4.0‰	0.53381 (0.00049) -1.5‰	0.50398 (0.00046) +2.3‰	1.17797 (0.00098) +3.9‰	0.30462 (0.00034) +10.5‰
July 17, 1974	AML.H15.81	24	253	0.05358 (0.00010) -11.1‰	0.03774 (0.00006) -8.2‰	0.52405 (0.00025) -2.7‰	0.53338 (0.00042) -2.3‰	0.50350 (0.00042) +1.4‰	1.17863 (0.00096) +4.4‰	0.30398 (0.00030) +8.4‰
September 29, 1974	AML.H15.81	13	436	0.05335 (0.00004) +15.3‰	0.03770 (0.00004) -9.2‰	0.52319 (0.00018) -4.3‰	0.53375 (0.00018) -1.6‰	0.50430 (0.00018) +3.0‰	1.17891 (0.00045) +4.7‰	0.30446 (0.00016) +10.0‰
April 18, 1975	AML.H15.117	14	178	nm	nm	0.52363 (0.00040) -3.5‰	0.53430 (0.00042) -0.6‰	0.50463 (0.00048) +3.6‰	1.18077 (0.0015) +6.3‰	0.30449 (0.00030) +10.1‰
September 6, 1975	AML.H15.104	31	261	nm	nm	0.52306 (0.00045) -4.5‰	0.53368 (0.00048) -1.7‰	0.50395 (0.00044) +2.3‰	1.17761 (0.00081) +3.6‰	0.30423 (0.00035) +9.2‰
September 11, 1975	AML.H15.81	4	170	nm	nm	0.52305 (0.00057) -4.6‰	0.53350 (0.00027) -2.1‰	0.50389 (0.00060) +2.2‰	1.17870 (0.00085) +4.5‰	0.30418 (0.00054) +9.1‰
December 4, 1975	AML.H15.117	13	392	nm	nm	0.52305 (0.00020) -4.6‰	0.53357 (0.00021) -1.9‰	0.50415 (0.00023) +2.7‰	1.17887 (0.00033) +4.6‰	0.30453 (0.00015) +10.2‰
	Mean ratios			0.05354 -11.7‰	0.03781 -6.2‰	0.52334 -4.0‰	0.53371 -1.7‰	0.50406 +2.5‰	1.17878 +4.6‰	0.30436 +9.6‰
December 5, 1975	Dilution with normal Cd (2 µg)	18	330 ng + 2 µg	nm	nm	0.52514 (0.00016) -0.6‰	0.53480 (0.00018) +0.4‰	0.50340 (0.00019) +1.2‰	1.17442 (0.00034) +0.9‰	0.30213 (0.00016) +2.3‰
	Laboratory standard ratio			0.05418	0.03805	0.52545	0.53461	0.50280	1.17342	0.30145

*The ratios are not corrected for instrumental discrimination. The error associated with each measurement (in parentheses) is two standard errors of the mean of sets of 10 ratios. The — difference between each isotope ratio and that for laboratory standard is given below the error, where — difference is defined for the 116/112 isotopic ratio as $[1 - (116/112)_{\text{sample}} / (116/112)_{\text{standard}}] \times 1,000$

The "laboratory standard" ratios are based on measurements on Cd from Cd-rich terrestrial minerals as well as repeated measurements of laboratory reagent Cd (Johnson Matthey Spec-pure metal).

†These figures are calculated from a knowledge of the weight of meteorite used and the cadmium concentration. No allowance has been made for the efficiency of the chemical extraction procedure which is ~ 50%. The chemistry blank for the separation is ~ 3 ng. In the dilution experiment 2 µg of reagent cadmium was added to the meteorite sample before dissolution.

‡AML, American Meteorite Laboratory, Denver, Colorado.

nm, No measurement made.

Cd isotope abundance in terrestrial samples² and a range of meteorites. The meteorite data will be reported in detail elsewhere. The Brownfield H3 chondrite, included in the survey because of its enhanced Cd content³, was found to contain anomalous Cd; some results are given in Tables 1 and 2.

Table 1 shows the Cd to be consistently isotopically fractionated relative to the laboratory standard, with an enrichment in the heavier isotopes. Figure 1 shows that a linear trend exists when the ‰ differences between the mean of the Brownfield data and the laboratory standard are plotted. The fractionation decreased by the expected amount when the meteorite Cd was diluted with reagent Cd (see Fig. 1).

Two samples of Brownfield were double spiked² with a ¹⁰⁶Cd-¹¹¹Cd tracer. The ratios measured for the mixtures as well as other data needed for the calculation of the fractionation are given in Table 2. Both mixtures yielded a fractionation of 2.3‰ per mass unit relative to laboratory reagent Cd (Johnson Matthey Spec-pure metal). Measurements made in this labora-

tory² have shown that the reagent Cd used for the experiment has the same isotopic composition as Cd from Cd-rich terrestrial minerals. There is excellent agreement between the double-spike result and the unspiked measurements (Fig. 1).

The data presented here so far show that recognised sources of isotopic discrimination such as chemical processing and mass spectrometry could not be the cause of the observed isotope fractionation. The fractionated Cd could still have a terrestrial origin, however, since Brownfield is a "find"⁴. To establish that the anomalous Cd was most probably present in Brownfield when it fell, it was necessary to find isotopically similar Cd in a meteorite known to be a "fall". A sample of the H3 chondrite Tieschitz was obtained for this purpose and the results of the analyses are given in Table 3. The Cd in Tieschitz is also clearly fractionated, although the results of the two measurements differ.

Further double-spike measurements indicate that both the fractionation and the Cd concentration are extremely variable

Table 2 Double-spiked data for Brownfield

Sample	No. of data sets	106/111*	110/111	116/111	Fractionation relative to: double spike reagent Cd (‰ per mass unit)
DS Brownfield	15	0.28183 (0.00029)	0.32864 (0.00021)	0.18720 (0.00019)	+1.3
DS Brownfield	33	0.26880 (0.00019)	0.37558 (0.00022)	0.21484 (0.00014)	+1.3
DS laboratory reagent	25	0.31491 (0.00039)	0.21237 (0.00022)	0.11684 (0.00010)	-1.1
DS laboratory reagent	22	0.27180 (0.00025)	0.36802 (0.00016)	0.20736 (0.00024)	-1.0
Double spike (DS)		0.36985 (0.00042)	0.02294 (0.00006)	0.00672 (0.00003)	

*See Table 1.

Table 3 Isotopic data for Tieschitz†

Unspiked	110/112*	111/112	113/112	114/112	116/112
No. of data sets = 9	0.52321	0.5335	0.5038	1.1785	0.30532†
Sample size = 50 ng	(0.00080)	(0.0011)	(0.0014)	(0.0021)	(0.00080)
Classification H3	-4.3‰	-2.1‰	+2.0‰	+4.3‰	+12.8‰
Double spiked	106/111	110/111	116/111	Fractionation relative to reagent cadmium (‰ per mass unit)	
No. of data sets = 18	0.33170	0.15260	0.08273	+1.5	
	(0.00021)	(0.00010)	(0.00006)		

*See Table 1.

†Source of sample: Dr G. Kurat, Vienna Museum for Natural History.

‡The observed enrichment beyond that expected for linear isotope fractionation is probably due to ^{116}Sn contamination. Such interferences may be resolved when a double spike is added by selecting 114 instead of 116 for computations.

in this meteorite. This may be due to dilution of a fractionated Cd component in the meteorite with an unfractionated component. It is also interesting to note that the Cd content of Tieschitz (values of 75–203 ng g⁻¹ have been obtained so far) is substantially lower than that in Brownfield (408 ng g⁻¹) but unusually high when compared with other H chondrites³. The chemical inhomogeneity is not unexpected owing to the primitive nature of the H3 chondrites. (Further analyses suggest that the concentration of 408 ng g⁻¹ for the bulk meteorite is more representative of Brownfield than that previously reported by us³.)

Linear isotope fractionation has only been detected in two of the 22 meteorites from various classes examined in this study.

At present we can only speculate on the origin of the anomalous Cd. Arrhenius and Alfvén⁵ have indicated several mechanisms which could lead to isotope fractionation during the formation of the Solar System and one of these may account for the observed anomaly. For instance, in chemical species with high re-emission coefficients, such as Hg and the noble gases, efficient isotope separation can be achieved by a multiple desorption process involving impingement, diffusion and desorption from the solid surfaces of a growing condensate. Arrhenius and Alfvén⁵ imply that the Hg anomaly observed by Reed and Jovanovic⁶ could be attributed to this mechanism. Although Brownfield was not measured by Reed and Jovanovic, it is interesting to note that Tieschitz yielded a 510‰ enhancement in $^{202}\text{Hg}/^{196}\text{Hg}$ ratio. Since Cd has a high ionisation

potential and is also volatile, this mechanism may also lead to isotope fractionation of Cd.

Lewis *et al.*⁷ have reported the presence of fractionated noble gases in chromite from Allende. Ne, Ar and Kr were enriched in the heavy isotopes whereas Xe was enriched in the lighter isotopes. They suggest that fractionation may have occurred during trapping of the gases from the solar nebula. An examination of Cd in chromite may therefore be profitable.

We thank Dr M. P. Gorton for discussions and Dr G. Kurat for providing the sample of Tieschitz. This research was supported by the Australian Research Grants Committee.

KEVIN J. R. ROSMAN
JOHN R. DE LAETER

Department of Physics,
Western Australian Institute of Technology,
South Bentley, Australia

Received February 24; accepted March 26, 1976.

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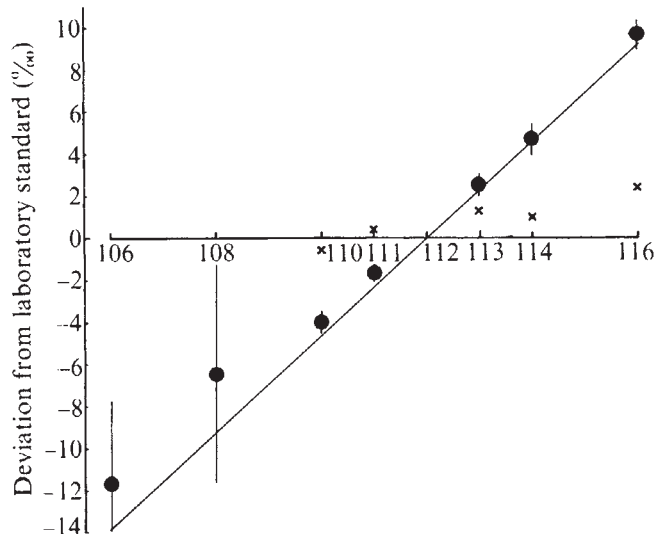
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Fig. 1 Isotope data for the Brownfield chondrite. ●, Mean of the unspiked analyses shown in Table 1. The error limits indicate two standard errors for the mean. ×, Result of diluting a sample of Brownfield with laboratory reagent Cd. The line represents a fractionation of 2.3‰ per mass unit which is obtained by independent measurements using a double-spiking technique.



Evidence for two discrete centres in Skye

ONE of the best-developed igneous areas in North-West Britain is on the Isle of Skye: in addition to extensive flood basalt lavas and their feeding dykes there are three adjacent intrusive centres across the south-east of the island¹. The Eastern and Western Red Hills, usually identified as two almost contemporaneous centres, comprise surface granite outcrops in a mass 10 km across, intruded against the older basic-to-ultrabasic Cuillin centre which is of similar size and lies to the west (Fig. 1a). Using aeromagnetic, gravity and isotopic evidence we will argue that there are two structurally discrete centres at depth, which probably were intruded several million years apart.

Bott and Tuson² have reported a large gravity anomaly over the Skye centres of ~50 mgal above the regional value (Fig. 1b). They interpreted this as due to a conical mass of gabbro widening down to a depth of 15 km, underlying all three centres and outcropping as the Cuillin, with the granitic Red Hills being no more than a veneer, perhaps 2 km thick. The aeromagnetic map³ shows, however, a negative anomaly of ~600 nT over the Cuillin, and a roughly equal but positive one over the Red Hills. Though the former can be accounted for if the Cuillin have a reversed remanent magnetisation (common in the province^{4,8,18}) the latter cannot be identified with the Red Hills because the anomaly is no less prominent on the filtered aeromagnetic map⁸ (Fig. 1c) which only registers bodies deeper than ~5 km in the Skye area⁶. We therefore

propose that there are two basic masses underlying the centres of Skye, having opposite magnetic polarities but sufficiently close together to appear as a single gravity anomaly.

It is inherent in our postulate (twin deep-seated masses which cannot be resolved by gravity) that a detailed interpretation is not possible with the present data. Indeed, simple calculations assuming two deep vertical gabbro 'cylinders' of 16-km radius in contact, as suggested in cross section by the magnetic map (Fig. 1c), have shown that only a single gravity anomaly would be resolved. To satisfy the observed magnetic anomalies we must assume that the induced magnetisation in these cylinders is small compared to their remanence⁷. If the latter has an inclination of 60°N, appropriate for an early Tertiary age⁸, a strong positive anomaly would be observed to the south and a weak negative to the north at the Red Hills, conversely for the reversely magnetised Cuillin root. Only the strong anomalies can be identified clearly in Skye, but in spite of this it is clear that the magnetic anomalies can be satisfied only if two opposite magnetised bodies are present.

Comparable strong positive and negative magnetic anomalies are not observed on the filtered maps for the other Tertiary centres, and the question arises in what way Skye differs. One possibility is that Skye contains centres formed at two distinct times. Field evidence shows that the succession commenced with the flood basalts; then followed the Cuillin centre which coincided with the later lavas, and in turn was succeeded by the bulk of the (basic) dyke swarm; last came the Western, then the Eastern, Red Hills, but there is no field evidence to determine the intervals in the succession, which have been assumed to be short⁹. Rb-Sr isochron dates on the Red Hills are 52 ± 3 Myr (ref. 10) and 51 ± 4 Myr (ref. 11), and all the 24 K-Ar dates of Kelly¹² are <55 Myr, except for two (59.2 ± 0.7 , 59.3 ± 1.0 Myr). There is no evidence for a detectable difference in age between the Eastern and Western Red Hills. The Cuillin is undated, and the only other relevant dated bodies are the lavas, for which the data are equivocal. Kelly¹² found that most of his lava samples also gave K-Ar ages <55 Myr. It is, however, believed from oxygen isotope work¹³ that the southern part of Skye was subjected to hydrothermal alteration caused by the heat of the centre or centres, and it may be significant that the one sample which came from a locality where the oxygen isotope ratio was normal, gave an apparent age of 59 Myr. Unpublished data from this laboratory on lava samples from the north of Skye also include some samples of higher ages. Thus a possible interpretation of the radiometric evidence is that the lavas—and therefore the Cuillin centre—were intruded ~ 59 Myr ago, but were extensively 'overprinted' by the heat and chemical changes associated with the Red Hills centres which were not emplaced until ~ 53 Myr ago. Such conditions probably would not have remagnetised the adjacent rock¹⁴ and, anyway, the magnetism in question here is deep seated.

This date for the lavas gains support from indirect evidence: the dispositions of the dyke-swarms of Skye, Mull and Arran strongly suggest that they were contemporaneous^{15,16}. The dykes of Mull mostly pre-date the Loch Bà centre (60.4 ± 1.9 Myr, Rb-Sr isochron¹⁷), whereas those of Arran came between the closely contemporaneous centres (Northern Granite 58.8 ± 0.6 Myr: Central Complex 58.3 ± 2.2 Myr, dated by the $^{40}\text{Ar}/^{39}\text{Ar}$ method¹⁸) and Holy Island (54.9 Myr, Beckinsale, unpublished); all these dates are consistent with the Skye dykes—and hence the Cuillin which preceded them—being ~ 59 Myr old.

It is a basic assumption of this paper that the Cuillin 'cylinder' acquired its remanence during a period of reversed polarity; an age of 59 Myr is consistent with this, for the lavas of Mull are of similar age and are reversed^{19,20}.

Strontium and lead isotopic evidence^{11,21} suggest that

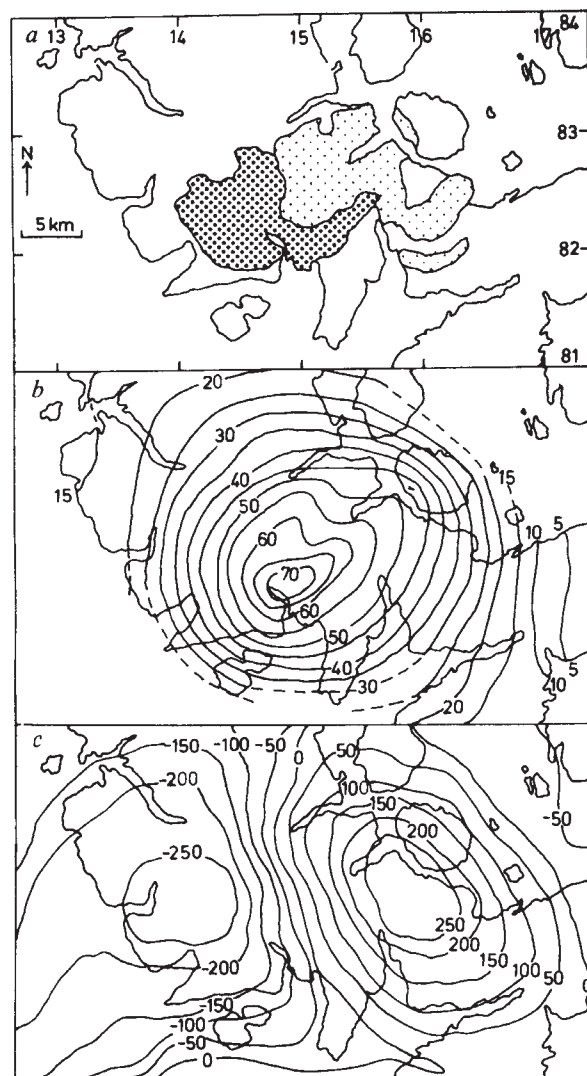


Fig. 1 a, Map of southern Skye showing the juxtaposition of intrusive centres at outcrop level¹. Distances are given in tens of kilometres north and east of the national grid origin. Dotted pattern, Cuillin gabbro; crossed pattern, Red Hills granite; blank areas, Tertiary lava and older rocks. b, Gravity anomaly² on the same scale with contours at 5-mgal intervals. c, Total field aeromagnetic anomalies filtered at 3.33-km intervals³, representing anomalous material buried beneath ~ 5 km (ref. 6). Contours at 50-nT intervals.

the Red Hills granites were mainly derived by partial melting of Lewisian rocks although the possible effects of hydrothermal alteration¹³ throws the isotopic evidence into doubt. Whether these granites were derived by partial melting, or by fractional crystallisation of the underlying basic rock mass, a large heat source is implied. This could not be the mass underlying the Cuillin if it were intruded several million years before; therefore, a second source is needed which we have identified from magnetic anomalies to be a second deep-seated basic mass which cooled during a normal polarity period.

G. C. BROWN
A. E. MUSSETT

Sub-department of Geophysics,
Oliver Lodge Laboratory,
University of Liverpool,
Liverpool L69 3BX, UK

Received December 15, 1975; accepted April 7, 1976.

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Promotion and retardation of heat transfer by electric fields

I REPORT here on the effects of an electric field on heat transfer. The effects depend on the applied voltage, the shapes, number and locations of the electrodes, and the frequency of the electric field^{1,2}.

The vaporisation of water is enhanced remarkably in an electric field: at 12 °C, the rate increases by up to 40–50 times³. This phenomenon suggests that heat transfer may well be influenced by electric fields; the experiments reported here show this to be the case. The rate of heat transfer in a gas can be increased by up to 1.5 times, in liquids by up to 2.0 times, and in solids by up to 1.6 times, according to the positioning of the electrodes and the strengths of the electric fields.

Under the influence of electric fields another interesting phenomenon has also been observed: the rate of cooling can be retarded. If an electrode is located within material that is to be heated, cooling proceeds more slowly after the heat source is removed. Cooling takes place 1.5 to 2.0 times more slowly than in the absence of an electric field.

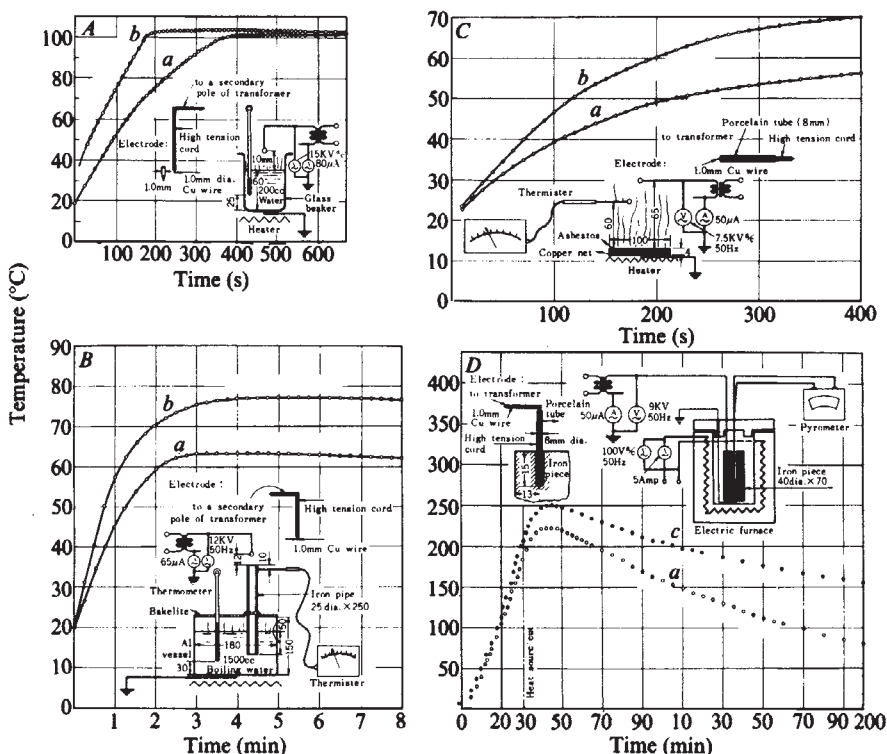


Fig. 1 A, Heating curves of 200 cm³ water contained in a glass beaker: *a*, in the absence of an electric field; *b*, in the presence of an alternating electric field (15 kV; 80 μA) with the electrode (Cu wire, diameter 1.0 mm) placed 10 mm above the water. Boiling point is attained twice as quickly in case *b*. B, Heating curve of an iron pipe (diameter 25 mm, length 250 mm): *a*, in the absence of an electric field; *b*, in the presence of an alternating field (12 kV; 65 μA) with the electrode (Cu wire, diameter 1.0 mm) placed 12 mm above the end of the pipe. The pipe was heated by placing its lower end in 1,500 cm³ of boiling water contained in an aluminium vessel insulated with a bakelite lid. In the presence of the electric field a temperature of 76 °C was obtained (*b*), compared with a maximum temperature of 63 °C with no electric field (*a*). C, Heating curves of free air: *a*, in the absence of an electric field; *b*, in the presence of an alternating field (7.5 kV; 50 μA) with the electrode (Cu wire, diameter 1.0 mm) placed 65 mm above a heated asbestos mat. Temperature of the air between the electrode and the mat reached 70 °C in case *b*, as opposed to 56 °C in case *a*. D, Heating and cooling curves of an iron cylinder (diameter 40 mm; height 70 mm): *a*, in the absence of an electric field; *b*, in the presence of an alternating field (9 kV; 50 μA) with the electrode (Cu wire, diameter 1.0 mm) inserted 15 mm into the cylinder. The cylinder was heated within an electric furnace, and in case *b* cooling was 1.6 times as slow as in case *a*.

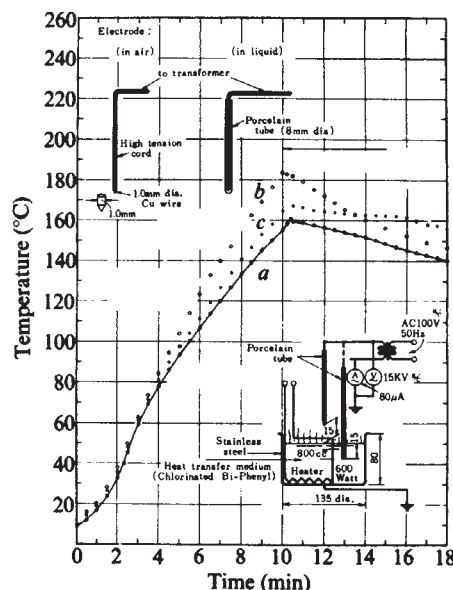


Fig. 2 Heating and cooling curves of chlorinated biphenyl (liquid) contained in a stainless steel beaker (diameter 135 mm), with the heating source placed within the liquid: *a*, in the absence of an electric field; *b*, in the presence of an alternating field (15 kV; 80 μA) with the electrode (Cu wire, diameter 1.0 mm) outside the liquid; *c*, in the presence of an alternating field (15 kV; 80 μA) with the electrode (Cu wire, diameter 1.0 mm) inside a porcelain tube within the liquid. After heating for 10 min, temperatures of 161, 184, and 167 °C were attained in cases *a*, *b*, and *c*, respectively. When the heat source was removed (after 10 min) the temperatures fell in 8 min to 140, 146, and 156 °C, respectively, in cases *a*, *b*, and *c*. In cases *b* and *c* the alternating current remained on, and cooling was 1.7 times slower (*b*) and 1.8 times slower (*c*) than in case *a*.

The restriction of heat transfer can be compared to the behaviour of current flow in an electrostatic transistor.

The methods and apparatus used in the experiments are described in Figs 1–3. It should be noted that no energy is absorbed from the electric field, except for 1.5–2.0 W as a result of ionisation or leakage.

If the nature of the promotion of heat transfer is considered thermodynamically, it can be regarded as a phenomenon in which materials (fluids) change their qualities so as to absorb more heat energy from their surroundings or transfer more heat energy within themselves.

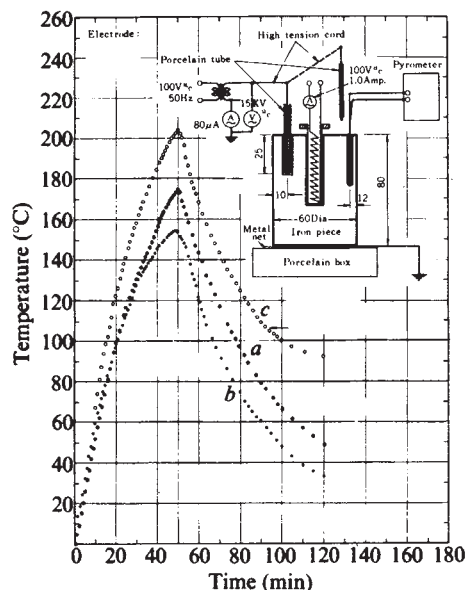


Fig. 3 Heating and cooling curves of an iron cylinder (diameter 60 mm, height 80 mm) with the heat source placed within the cylinder: *a*, in the absence of an electric field; *b*, in the presence of an alternating field (15 kV, 80 μ A) with the electrode outside the cylinder; *c*, in the presence of an alternating field (15 kV, 80 μ A) with the electrode (Cu wire, diameter 1.0 mm) inserted 25 mm into the cylinder. After heating for 50 min, temperatures of 175, 155, and 205 $^{\circ}$ C were attained in cases *a*, *b*, and *c*, respectively. In this case, when the heat source is removed cooling is more rapid in case *b* than in case *a*; a similar phenomenon occurs with liquids, however, if only small quantities of heat are supplied initially.

The phenomenon could be investigated further through studies of infrared absorption patterns⁴ and increases of vapour pressures^{5,6}.

Y. ASAKAWA

*The Japan Society of
Mechanical Engineers,
Sanshui Hokusei Building,
Shibuya-Ku, Tokyo 151*

Received October 20, 1975; accepted March 11, 1976.

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Microfossils from a newly discovered Precambrian stromatolitic iron formation in Western Australia

THE Nabberu Basin is a newly discovered sedimentary basin of probable early Proterozoic age in Western Australia^{1–3} (Fig. 1). It covers an area of $\sim 60,000$ km², and contains $\sim 6,000$ m of shallow-water clastic and chemical sediments. It is essentially unmetamorphosed in the south-east but is metamorphosed to granulite facies in the west, where folding and deformation are more pronounced.

In the middle of the Nabberu Basin sequence is the Frere Formation— $\sim 2,000$ m of alternating banded iron

formation and shale, with thin stromatolitic dolomite at its base. The iron formations are granular and are very similar to those of the Animikie Basin and Labrador Trough in North America^{4,5}. Within the Frere Formation at a location of about 2.5 km north-east of Camel Well in the south-eastern part of the basin (latitude $26^{\circ}08'$, longitude $121^{\circ}18'$), a thin oncolitic iron formation is interbedded with peloidal and oolitic ferruginous chert. The oncolites and associated stratiform stromatolites contain the abundant microfossils^{6,7}. Microfossils, as yet unstudied, are also present in the Windidda Formation, overlying the Frere Formation, in thin, black to grey apatitic and sulphidic cherts near Tooloo Bluff (latitude $26^{\circ}27.5'$, longitude $122^{\circ}14'$).

The iron formation microfossils are of special interest because the assemblage is almost identical to that of the Gunflint Iron Formation of Ontario⁸, but is different in many respects from other described Precambrian assemblages⁹. This similarity may result from the facies similarity or from the probable pencontemporaneity of the two deposits, which we shall discuss. This is the first time that two particularly distinctive and comparable assemblages of Precambrian microfossils have been found widely separated on two continents and the biostratigraphic significance of this discovery must be assessed. The nature of the occurrence of microfossils within oncolites indicates a benthonic mode of life, conflicting with an interpretation¹⁰ of a planktonic mode for several of the same taxa from the Gunflint. Here we briefly describe the Nabberu Basin microfossils and consider the questions and comparisons outlined above.

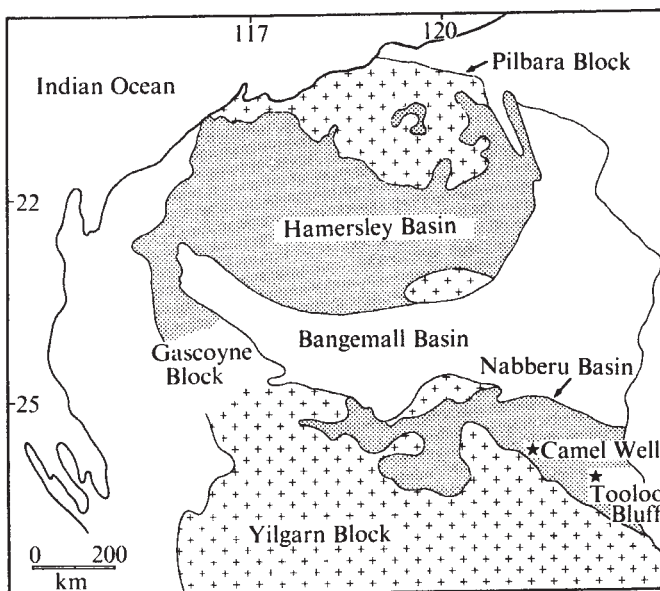


Fig. 1 Locality map. Crosses represent Archaean rocks, stippled areas are early Proterozoic sedimentary basins; the Bangemall Basin is Middle Proterozoic; the remaining unornamented areas are underlain by Phanerozoic rocks.

The Nabberu sediments unconformably overlie the Archaean granite/greenstone basement of the Yilgarn Block, and are unconformably overlain by sediments of the approximately 1,100-Myr-old Bangemall Basin (Fig. 1). Glaucconites from the basal Yelma Sandstone of the Nabberu Basin give an average minimum age of 1,700 Myr (ref. 3), which is about the time of tectonism of the nearby Hamersley Basin. There is evidence to suggest that the Nabberu Basin can be traced through the high grade Gascoyne Block at its western extremity into the sediments of the Hamersley Basin, which are well dated as approximately 2,000 Myr old¹¹. Thus the Frere Formation of the Nabberu Basin seems to be approximately the same age as the Gunflint Iron Formation.

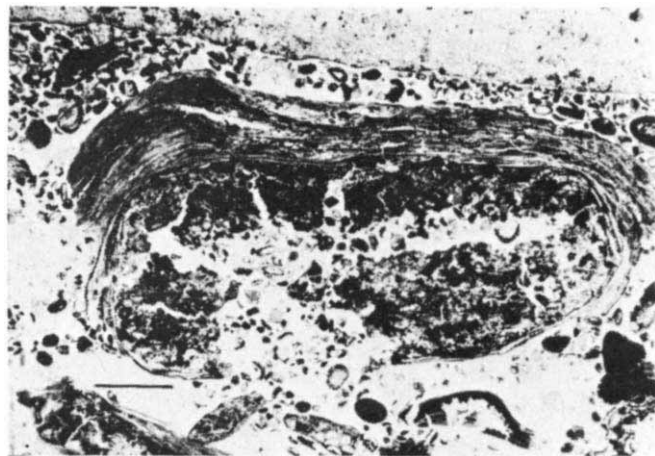


Fig. 2 Microfossiliferous oncolites and granules, Frere Formation. Scale bar represents 2 mm. Thin section BHP 5319D3. All specimens are kept in the Bureau of Mineral Resources, Geology and Geophysics, Canberra.

The microfossils occur within discoidal and subspherical stromatolites (that is, oncolites), stratiform stromatolites, and large granules (peloids) (Fig. 2). Filaments 1–2 μm wide and up to hundreds of microns long (here identified as *Gunflintia minuta* Barghoorn) are abundant and ubiquitous in the stromatolites (Fig. 3, *a* and *b*), and most are contorted and randomly oriented. Not uncommonly, however, groups of filaments are aligned roughly parallel to the stromatolite laminae, and occasionally such groups can be followed into contiguous laminae where they turn to become approximately perpendicular to the laminae. The organisms represented by these filaments seem to have constructed the stromatolites. The filaments, which are preserved as segmented rods of iron oxide, sometimes occur within tubes of iron oxide (Fig. 3*c*). These and other non-septate tubes 4–9 μm wide (Fig. 3*d*) without internal filaments (trichomes) are interpreted as the former mucus sheaths of cyanophytes, as is *Animikiea septata* Barghoorn from the Gunflint¹⁰; the Frere Formation tubes have a slightly different range of sizes and are less clearly annulate than the Gunflint forms and are therefore referred to a new species of *Animikiea*.

Rosettes of filaments occur singly and in clusters within the stromatolites and granules (Fig. 3*e–g*). Each rosette consists of 5–20 filaments of variable width (0.5–1.5 μm); the width of the rosettes is 5–25 μm . The filaments are unbranched (here referred to *Eoastrion simplex* Barghoorn) or, rarely, dichotomously branched (referred to *E. bifurcatum* Barghoorn). The degree of mineralisation of the filaments is variable; they are often heavily encrusted with iron oxides and so coalesce into a subspherical central body. They closely resemble the extant benthonic iron- and manganese-precipitating bacterium *Metallogenium personatum*¹², as has been noted previously¹³.

Hollow spheres and ellipsoids of iron oxide referable to the genus *Huroniospora* Barghoorn occur singly or, more commonly, in groups with separate individuals (Fig. 3*h–k*). They range in width from 2.5 to 12.0 μm , with a mode and a mean of 6 μm . There are several examples of apparently dividing cells (Fig. 3*i* and *j*). The three species originally described from the Gunflint were distinguished by their wall textures and thicknesses, features that are now considered as possibly secondary¹⁰. The species have not yet been redefined. It is notable, however, that many of the cells in the Frere Formation resemble those in the Gunflint originally called *H. psilata* Barghoorn.

One of the most distinctive microfossils in the Gunflint is *Kakabekia umbellata* Barghoorn. This is also present, but relatively rare, in the Frere Formation (Fig. 3*l–o*), where it occurs separately or in small groups within the stroma-

tolites. An extant homoeomorph has been described from soils¹⁴. One example has been found of a structure that resembles the branched tubular fossil *Archaeorestis schreiberensis* Barghoorn from the Gunflint, but identification must wait until more samples have been discovered.

In the Gunflint Iron Formation there are two different microfossiliferous chert facies: "One, a thin bedded chert, contains what is interpreted as a planktonic assemblage of microorganisms dominated by *Kakabekia umbellata* Barghoorn, *Eoastrion simplex* Barghoorn, and *E. bifurcatum* Barghoorn"¹⁰. The other is stromatolitic, and thus benthonic, and is dominated by *Gunflintia minuta* and *Huroniospora* spp., with rarer specimens of *G. grandis* Barghoorn, *Animikiea septata* and other forms. The Frere Formation stromatolitic assemblage described here contains

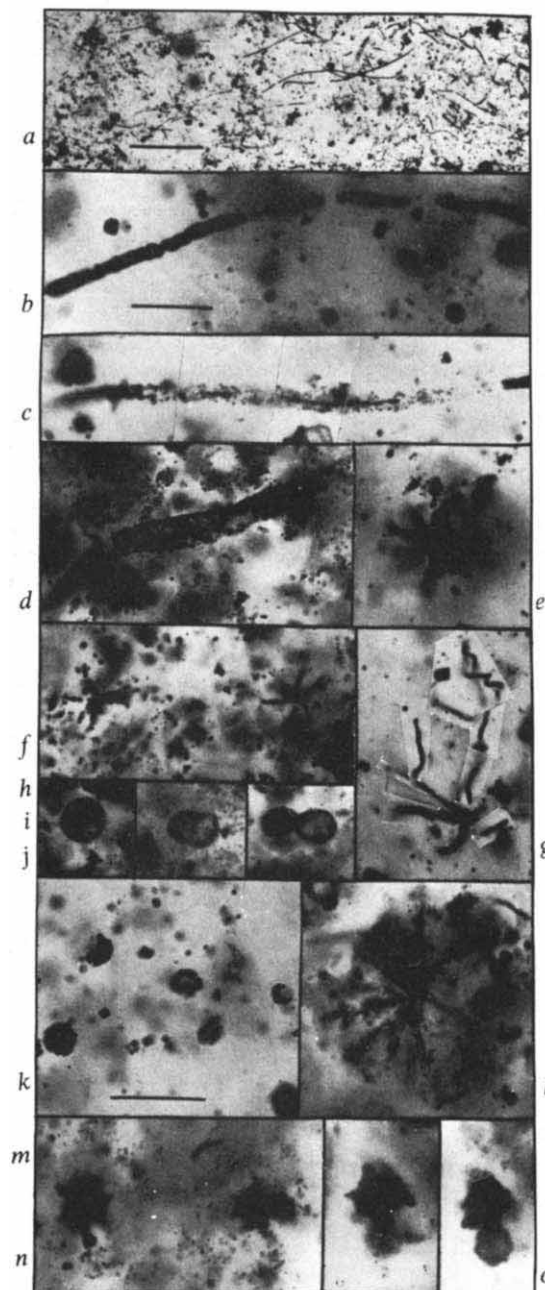


Fig. 3 Microfossils from the Frere Formation. All photographs are of petrographic thin sections of ferruginous chert in transmitted light. Scale bar in *a*, 100 μm ; in *b*, 10 μm ; in *k*, 20 μm (that in *k* applies to photographs *c–o*). *a* and *b*, *Gunflintia minuta*; *c* and *d*, *Animikiea* sp. nov., in *c* with an internal *G. minuta* trichome; *e*, *f* and *g*, *Eoastrion simplex*; *h–k*, *Huroniospora* spp.; *l–o*, *Kakabekia umbellata*, photographs *n* and *o* are of the same fossil at 2 different focal levels. All are in thin section BHP 5319D1.

representatives of both these Gunflint assemblages: it resembles the Gunflint stromatolite assemblage in that it is also dominated by *G. minuta* and *Huroniospora* spp., but in addition contains abundant *E. simplex* and rare *E. bifurcatum* and *K. umbellata*, considered by Awramik¹⁰ to have been planktonic (although the basis for this interpretation has not yet been published). The occurrence of *E. simplex* in clusters within the Frere Formation oncolites strongly suggests that at least in the Nabberu Basin this organism was benthonic, an interpretation that is consistent with the fact that the most similar extant organism, *Metallogenium personatum*, inhabits lacustrine muds¹². Furthermore, at least some extant rosette-forming bacteria attach to a substrate before forming rosettes¹³. Similarly, *K. umbellata* may also have been benthonic since it occurs in clusters and the only known extant homoeomorph inhabits soils. The search for microfossils in non-stromatolitic laminated cherts of the Frere Formation has been so far unrewarded, and only dubious microfossils have been reported¹⁶ from the possibly correlative non-stromatolitic, non-granular iron formations of the nearby Hamersley Basin.

The stromatolites of the Gunflint Iron Formation and the Frere Formation seem likely to have formed in very similar environments. Both are composed of almost certainly primary silica and iron compounds and occur with granules and ooids which suggest a turbulent sub-aqueous environment. These palaeoenvironmental similarities may be sufficient to explain the near identity of the two assemblages of microfossils and their differences from older and younger assemblages^{7,17}, since these older and younger assemblages are almost entirely from fine grained siliciclastic rocks and secondary cherts in stromatolitic carbonates. Such a close similarity of two probably penecontemporaneous microfossil assemblages, however, certainly encourages further investigation of the biostratigraphic usefulness of Precambrian microfossils. It is not possible to consider this aspect in detail here, but it is evident that the discovery of the Frere Formation microbiota will prove important in studies which use chert microbiotas in Precambrian biostratigraphy.

We thank the Director, Bureau of Mineral Resources, Geology and Geophysics, and the General Superintendent, Exploration, of the Broken Hill Proprietary Co. Ltd, Australia for encouragement, and J. H. Oehler for critically reading the manuscript.

M. R. WALTER

Bureau of Mineral Resources, Geology and Geophysics,
PO Box 378,
Canberra City ACT 2601,

A. D. T. GOODE

Melbourne Research Laboratories,
The Broken Hill Proprietary Co. Ltd,
Wellington Road,
Clayton, Victoria 3168,

W. D. M. HALL

The Broken Hill Proprietary Co. Ltd,
140 William Street,
Melbourne, Victoria 3000,
Australia

Received February 2; accepted March 18, 1976.

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Population genetic, social, linguistic and topographical relationships in north-eastern Arnhem Land, Australia

GENETIC differentiation has been found among Australian Aboriginal tribes¹, but no previous attempt has been made to assess intratribal differentiation. We report here on genetic differentiation within an Aboriginal 'tribe' related to linguistic, social and topographical components of the population, together with confirmation of the importance of drainage systems as natural barriers to gene flow. An estimate of inbreeding in a traditionally structured Aboriginal local group is given.

In an earlier intertribal study¹ we found that genetic differentiation was highly correlated with linguistic and sociocultural divisions. More recently we have concentrated on genetic differentiation at the level of the territorial and marriage unit within the Yolngu (a collective term for the dialects of the region (B. Schebeck, personal communication)) of the north-eastern Arnhem Land region of the Northern Territory of Australia (Fig. 1). This region is of considerable anthropological importance^{2–4}. It is one of few remaining areas of Aboriginal Australia where the simultaneous effect of ecological, demographic, sociocultural, linguistic and biological influences on phenotypic variation can be investigated. The Yolngu population is consistent with a biological definition of tribe⁵ being at least 90% endogamous. They are organised into localised totemic clans and dialect units which are territorially based and exogamous⁴. They now number about 2,400 and occupy a territory of about 26,000 km², bounded by coastline to the north and east and river systems to the west and south (Fig. 2).

This report concerns two dermatoglyphic traits, fingerprint pattern index and total ridge-count, both strongly heritable⁶. In addition, phenylthiocarbamide (PTC) taster ability, red-green colour blindness, handedness, earlobe form, hairy pinnae and skin colour were studied and generally supported our findings from the dermatoglyphic

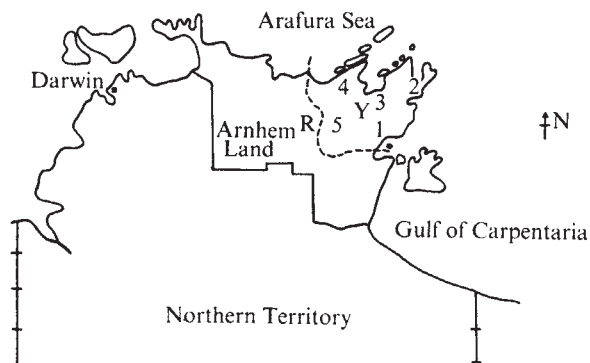


Fig. 1 Map of Arnhem Land, Northern Territory of Australia, showing the distribution of the Yolngu (Y) and Rembarranga (R) 'tribes', and the approximate location of marriage clusters (1–5) within the Yolngu. The dashed line shows the approximate boundary of the Yolngu.

Table 1 The relationship of the various population units to the genetic structure of the Yolngu, as assessed by dermatoglyphics

Unit of comparison	No. used in analysis	No. of comparisons	No. significantly different at probability level*				Percentage of comparisons significant at ≤ 0.05
			0.05	0.02	0.01	0.001	
Dialect groups	7	21	—	2	1	2	24
Ceremonial/'war' groups	6	15	3	1	—	—	27
Marriage clusters	5	10	3	2	1	—	60
Drainage divisions	6	15	—	4	1	7	80

*Pairwise *t* comparisons.

data. A total of 744 Yolngu were sampled, that is, 31% of the population. In addition to biological information, data were obtained on the social affiliation of the parents and spouse(s) of each subject, their ages and places of birth, together with information on marriage networks, genealogies and the distribution of the territorial units.

Table 1 indicates some genetic differentiation among the dialect groups of the Yolngu. Nine such groups, of which we have adequate data for seven, each consisting of a number of dialect units, are recognised by both linguist and Aboriginal, all but two being potentially endogamous⁷. Each statistically significant comparison involved the same dialect group, which suggests that the dialect groups form an intermarrying complex with no genetically significant endogamy within them, although there is some support for the suggestion of two linguistic "divisions" among the dialect units of the Yolngu⁸.

Total ridge-count was analysed according to dialect unit affiliation within recognised ceremonial/fighting groups. These groups were said by Aboriginal informants to be "tied together for maridjama (fighting) and manikaj (ceremonial singing)". The analysis revealed statistically significant differences, all of which were accounted for by the 'fighting' group known as the Balamumu, a collection of dialect units in the Blue Mud Bay area (Fig. 2). This suggests that the Balamumu, at least, functions largely as an endogamous unit, being genetically distinguishable.

Marriage 'clusters' were recognisable from the marriage data and correspond to those dialect units among which there was at least 70% endogamy. The dialect units that intermarried most generally had adjoining territories.

There were similarities between the structure of the ceremonial/fighting groups and marriage 'clusters', suggesting that the former groups are based on marriage relationships. The marriage cluster centred on the Blue Mud Bay region (Fig. 1, cluster 1) was distinctive, an observation consistent with our findings for the Balamumu fighting group.

The marriage clusters are also associated with drainage divisions. Peterson⁹ highlighted the role of drainage basins in the cultural and social differentiation in Aboriginal Australia. He noted that watersheds might have acted as natural barriers to communication. Independently we considered the drainage basins as units for analysis: our results (Table 1) indicate that these natural divisions are important in terms of genetic differentiation. In this analysis, data are included from some other neighbouring tribes, as drainage basins overlap the Yolngu territory (Fig. 2).

For the dermatoglyphic traits the greatest and most statistically significant difference was between the Aborigines living in the Blue Mud Bay area, and those living at the western boundary of the Yolngu. These two groups are separated topographically by a complex of low ranges (Fig. 2) which almost certainly restrict intermarriage although important ceremonial links exist. There is also evidence for gene flow from some neighbouring tribes into the western Yolngu group. This no doubt increased the extent of the differences between the groups. We found for the fingerprint, and other biological traits, that the three Yolngu dialect units along the western boundary are not significantly different from their immediate western tribal neighbours, the Rembarrunga (Fig. 1). There is also no sharp linguistic boundary to the west (B. Schebeck, unpublished).

We concentrated on a traditionally structured local group in the western Yolngu region made up of about 60 people belonging mainly to two intermarrying dialect units. The average level of inbreeding for this local group was estimated to be 0.02 from genealogical data—a value similar to that for Central and South American Indian populations¹⁰. The highest individual coefficient of inbreeding was 0.07 from data over five generations.

In conclusion, the Yolngu function as a marriage network of endogamous units within a broad cultural and linguistic complex. At both the 'tribal' and regional level these endogamous units seem to be closely related to the drainage basin. This finding supports Warner's² description of "Murngin" (Yolngu) society as a "confederacy" of several "tribes", at least when the term 'tribe' is used in the biological sense.

This project was supported by the Australian Institute of Aboriginal Studies. We thank N. H. Scarlett, Dr D. Biernoff and several Aboriginal informants for field assistance and discussion, and the Aborigines who co-operated in the study.

N. G. WHITE
P. A. PARSONS

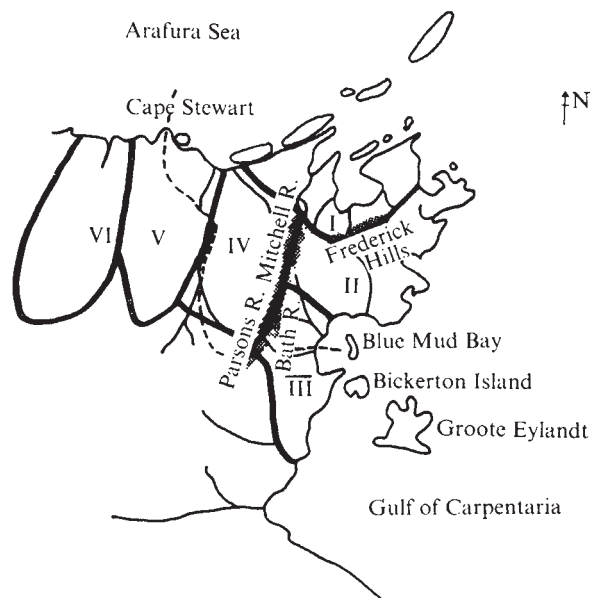
Department of Genetics and Human Variation,
La Trobe University,
Bundoora, Victoria 3083, Australia

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Fig. 2 Map of north-eastern Arnhem Land, Northern Territory, showing drainage divisions (I–VI) and some topographical features. Solid lines indicate major river systems, and the dashed line is the approximate boundary of the Yolngu.



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Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*

THE wood lemming (*Myopus schisticolor* Lilljeborg), a small rodent inhabiting mossy forests of northern coniferous areas of Eurasia, is unique with respect to sex ratio, showing a definite prevalence of females, and with respect to the occurrence of two types of female, one producing daughters only, the other producing progeny of both sexes^{1,2}. We thought that these phenomena might be associated with chromosome constitution, and as the chromosomes of this species were poorly known, we have made a detailed study of its karyotype. Some additional observations, pertinent both to the occurrence of chromosome variation and to reproduction, will be reported elsewhere. We report here two remarkable findings: (1) the existence of two types of fertile female with different somatic sex chromosome constitutions—the orthodox female complement, XX, and the male complement, XY, and (2) evidence for selective non-disjunction in XY females, leading to the formation of X-type egg cells only.

The material for the chromosome study consisted of 29 specimens collected in four areas of Sweden, and some 180 descendants of animals trapped in a fifth locality in Sweden (Table 1). Mitotic chromosomes were studied in direct preparations from bone marrow, and in fibroblast cultures from lung, heart or embryonic tissues. Cells were

Fig. 1 G banded chromosomes of *M. schisticolor*. *a*, Karyotype of a male. *b* and *c*, XY sex chromosomes of a female. *d*, XY sex chromosomes of a male. Scale = 10 μ m.

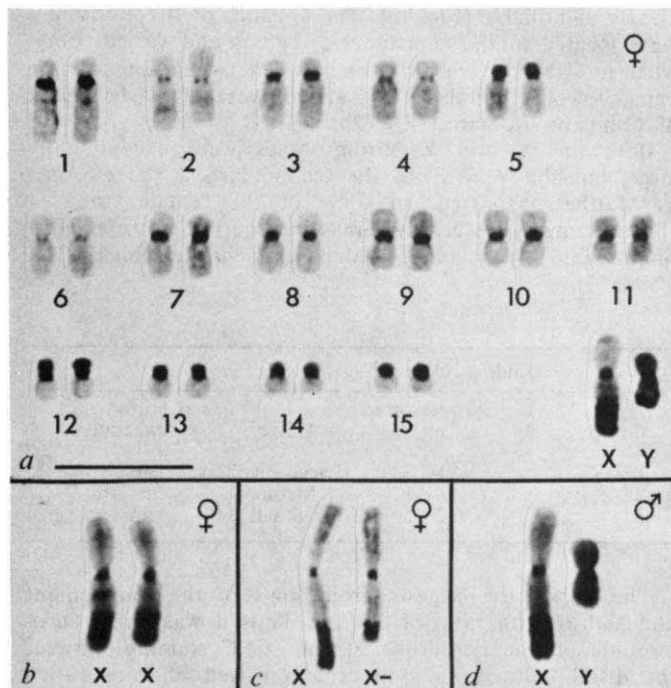
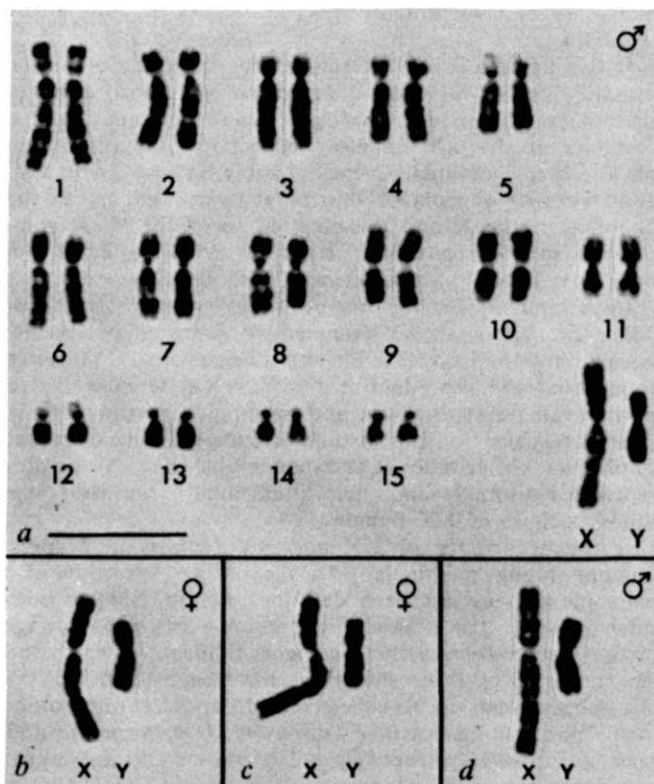


Fig. 2 C-stained chromosomes of *M. schisticolor*. *a*, Karyotype of an XY female. Note the absence of centromeric heterochromatin in nos. 2, 4 and 6. Pair no. 2 is dimorphic in this individual. *b*, XX sex chromosomes of a female. *c*, Sex chromosomes from a female with one normal and one deleted X. *d*, XY sex chromosomes from a male. Note the same staining pattern as in the XY female. Scale = 10 μ m.

usually processed by the conventional air drying procedure and the preparations were stained with orcein, Giemsa or the fluorochromes 33258 Hoechst and acridine orange. G and C staining^{3,4} were applied to preparations from most of the animals. The late replication pattern of the chromosomes was examined by autoradiography. Meiosis was studied in testes from eight males and in ovaries from 19 females. In males, meiotic chromosomes were studied by the method of Evans *et al.*⁵. For females the method described by Tsuchida and Uchida⁶ was used. Primarily diakinesis and metaphase I were evaluated. Histological sections of the female reproductive tract were prepared in the usual manner from most animals studied.

The chromosome number of the animals was $2n=32$ (Fig. 1), confirming Matthey's study⁷. The autosomes were divided into three groups on the basis of size and arm ratio (nomenclature according to Levan *et al.*⁸, Table 2). Chromosomes 5 and 12 had satellites attached to their short arms. After G staining, every autosome pair was

Table 1 Data on wood lemmings (*Myopus schisticolor*) from Sweden

Trapping locality and year	Animals studied		Sex chromosomes	
	Sex	No.	XY	XX
Våmhus, Dalarna (61°15'N; 14°15'E)	Male	—		
	Female	3*		3
Tandsjöborg, Dalarna (61°45'N; 14°30'E)	Male	1	1	
	Female	—		
Ärjäng, Värmland (59°15'N; 12°15'E)	Male	3	3	
	Female	4	4	
Hällnäs, Västerbotten (64°15'N; 19°45'E)	Male	5	5	
	Female	13	4	9
Näs, Dalarna (60°30'N; 14°30'E)	Male	17	17	
	Female	161	74	87

*Pregnant female with four embryos. Chromosome preparations were obtained from the mother and two of the embryos.

†The animals studied are from a colony kept by F.F. since 1963. They are descendants of five males, and ten females trapped at Näs.

clearly identifiable (Fig. 1a). The C bands of the autosomes were located in the centromeric regions and varied somewhat in size both within and between populations. Three pairs, however, numbers 2, 4 and 6, were regularly devoid of C heterochromatin (Fig. 2a).

Both the X and Y chromosomes were exceptionally large, but the Y was not the second largest chromosome as Matthey⁷ reported. In some of the females the sex chromosomes were XX, whereas in others they were indistinguishable from the male sex chromosomes, XY (Table 1).

Table 2 Autosomes of *M. schisticolor*

Group	Chromosomes no.	Size	Centromeric position
1	1-5	Large-medium	sm-st
2	6-11	Medium	m-sm
3	12-15	Small	sm-st

The X was the largest chromosome of the complement and had an arm ratio of 1.5-1.7. Thus it was an m chromosome on the borderline of sm. As C staining showed, the distal half of its long arm consisted of constitutive heterochromatin (Fig. 2a-d). It stained darkly by C staining, fluoresced comparatively weakly after staining with Hoechst 33258 and showed late DNA synthesis. Occasionally, a finer substructure of heavily stained bands was seen within the C segment. Sometimes on an X there were faint indications of gaps between the euchromatic and heterochromatic parts and within this latter segment. This observation was possibly related to occasional variations in the size of the heterochromatin: in one XY female it was about two-thirds, and in one X chromosome of one XX female it was only one-third of the normal size (Fig. 2c). The centromeric region of the X was C-band positive.

The Y was an m chromosome of medium size, similar to numbers 9-10 but with a somewhat higher arm ratio. These and the following features apply equally to the Y in males and to the Y-like sex chromosome in XY females. The entire Y was dark after C staining, clearly darker than the euchromatic parts of the X, although often less dark than the heterochromatic parts of the X. There were different intensities of staining within the Y, the distal part of the long arm being darkest. The centromeric region of the Y was not particularly heavily stained. The Y synthesised DNA late. Both Y and X showed characteristic G-band patterns, even though the bands in the entire Y, as well as in the distal half of the long arm of X, were less distinct than in the autosomes (Fig. 1).

C and G staining showed that the Y of the females was not a deleted or rearranged X chromosome. This is supported by the following evidence: (1) the 'female Y' always had exactly the same morphology as the 'male Y', including every detail of the banding pattern, and (2) the 'female Y' did not have any of the staining properties of the regular X chromosome.

Further clarification was obtained from an examination of meiosis. In male diakinesis the X and Y chromosomes were associated end-to-end (Fig. 3a). The sex bivalent, however, usually had a coiled configuration, not allowing distinct recognition of the two constituents. The structure of the 15 autosomal bivalents, on the other hand, was mostly clear and distinct. At diakinesis of XX females there were 16 symmetrical bivalents, 15 of them autosomal and one—the largest—formed by the two Xs (Fig. 3b). The XX bivalent had one or two chiasmata. In all 29 diakinesis figures from 10 XY females, the composition and type of bivalents was the same. Again the large sex-bivalent was clearly identifiable as a symmetrical figure

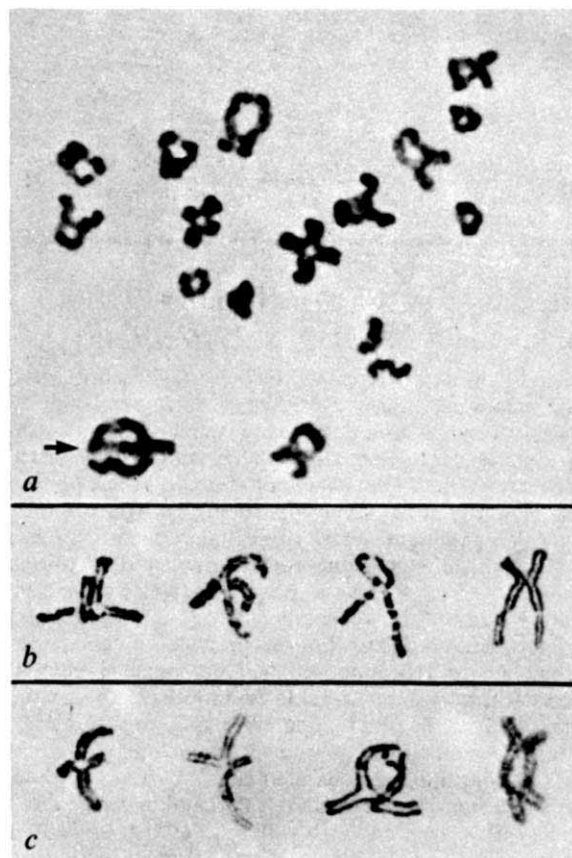


Fig. 3 Male (a) and female (b and c) meiosis in *M. schisticolor*. a, Complete cell in diakinesis showing 15 autosomal bivalents and the XY bivalent (arrow indicates the site of end-to-end association between X and Y). b and c, Sex bivalents from eight cells in diakinesis from XX (b) and XY (c) females (four and three different individuals, respectively). Note that in both XX and XY females the bivalent is symmetrical and composed of two Xs with one or two chiasmata.

with lateral pairing and one or two chiasmata and with no indication of structural or heterochromatic heterozygosity (Fig. 3c).

It has to be assumed therefore that the females with a somatic XY set possess an orthodox XX constitution in their oocytes. This discrepancy between the soma and the germline of the XY females necessitates the assumption of a non-disjunctional event, possibly in the primordial germ cells or oogonia of the foetal ovary, leading to the doubling of the X and the elimination of the Y. A comparable situation has been reported by Ohno *et al.*⁹ in *Microtus oregoni*, a species with XO females.

Both kinds of female, irrespective of whether they possessed two Xs or an XY complement, were phenotypically normal and indistinguishable from each other. Thus we found that the reproductive tract of XY females had a normal female appearance, and histological sections of the ovaries revealed follicles in different stages of development similar to XX females. Correspondingly, the XY females reproduce normally and their litters are of the same size order as those of XX females.

Is the occurrence of XX and XY females in *Myopus* unique among mammals? As far as we know such a phenomenon has not been described before, but we consider it likely that a similar condition exists in lemmings of the species *Dicrostonyx torquatus* (Pallas), in which the chromosomes of three subspecies have been described¹⁰⁻¹³. We suggest that the so-called x_1 , x_2 and x_3 chromosomes in the three subspecies *D. t. stewartsoni*, *D. t. torquatus* and *D. t. chionopaes*, respectively, all correspond to a Y chromosome, just as in *Myopus*. We postulate that both in

Myopus and in *Dicrostonyx* some of the XY animals function as fertile females due to an X-linked gene repressing the male-determining effect of the Y (our work in preparation).

This work was supported by grants from the Swedish Natural Science Research Council (K.F.) and the Deutsche Forschungsgemeinschaft (A.G.). We thank Miss G. Noack (Lübeck) for her help.

KARL FREDGA

Institute of Genetics,
University of Lund,
S-223 62 Lund, Sweden

ALFRED GROPP
HEINZ WINKING

Abteilung-für Pathologie,
Medizinische Hochschule Lübeck,
D-2400 Lübeck, FRG

FRIITZ FRANK

Biologische Bundesanstalt für Land-
und Forstwirtschaft,
D-3300 Braunschweig, FRG

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Epithelial origin of starfish coelomocytes

THE various theories proposed to explain the origin of echinoderm coelomocytes can be classified into two groups, depending on whether they refer to a cytopoietic organ or tissue (Tiedemann bodies¹⁻³, Polian vesicles¹, axial organ^{1,4}, connective tissue^{5,6} and coelomic epithelium⁷⁻⁹) or whether they imply self-replication of free cells⁹⁻¹¹. So far no theory has been confirmed. We now report that in the starfish (*Asterias rubens*) coelomocytes are produced in the coelomic epithelium.

A. rubens has only one type of coelomocyte¹², which, like the coelomic epithelial cells¹³, is easy to label by intracoelomic injection of ferritin (cadmium-free, Calbiochem, final concentration 3-5 mg ml⁻¹ of coelomic fluid). The iron is then made visible by applying Perls' reaction¹⁴ on smears and histological sections.

Cuénot's observations¹³ are correct: only the coelomocytes and the coelomic epithelial cells absorb ferritin. Injections have shown that there is no transfer of cells from one coelom to another (Table 1). Therefore, the

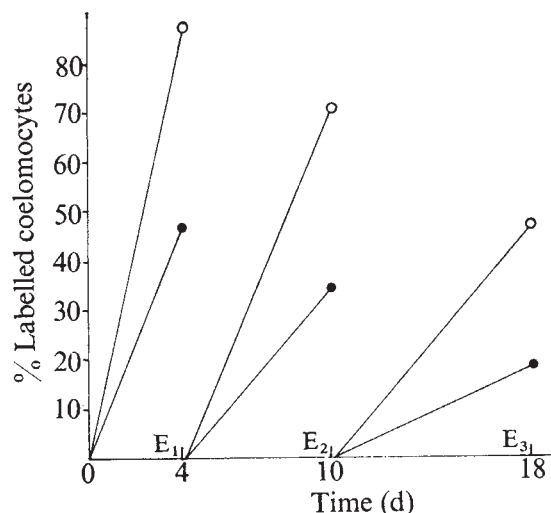


Fig. 1 Production of labelled coelomocytes at each bleeding (E_1 , E_2 and E_3) in two starfish (O, ●).

coelomic epithelium which lines the axial organ is not labelled unless ferritin is injected into the axial sinus. Also labelled coelomocytes are never found in the general body cavity after injection into the hydrocoel or the axocoel, and vice versa. Contrary to what happens in other echinoderms, the starfish's coelomocytes leave the coelom only to be eliminated into the external medium.

To determine the site of origin of the coelomocytes, two starfish which had been injected with ferritin (into the general body cavity) were bled several times. After the first bleeding, quite a large number of new coelomocytes

Fig. 2 Change in the number of iron-containing granules in 300 μ m of coelomic epithelium (pyloric caeca).

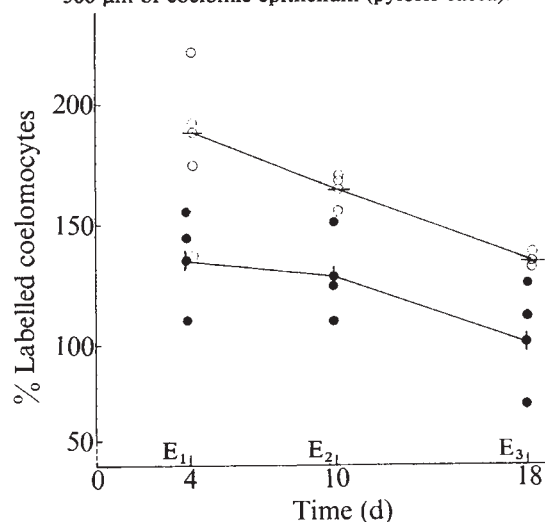


Table 1 Cellular labelling after intracoelomic injection of ferritin

Injected coeloms	Coelomocytes			Coelomic epithelia										Internal cavities of X and Y	
	A	B	C	Digestive tract	Papulae	Ambulacral ampullae		Tube feet	Tiedemann bodies		Axial sinus		Axial organ (X)		Gastric haemal tufts (Y)
				A	A	B	B	A	B	A	C	C	A		
Somatocoel	+	-	-	+	+	+	-	-	+	+	-	-	-	+	-
Hydrocoel	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-
Axocoel	-	±*	+	-	-	-	-	-	-	±*	-	+	+	-	-

A, Somatocoelic; B, hydrocoelic; C, axocoelic.

*Passage through the dorsal orifice between stone canal and axial sinus.

Table 2 Evolution of total number of coelomocytes ($\times 10^6$)

Starfish	T	4 d			6 d			8 d		
		N	M	V	N	M	V	N	M	V
No. 1		180*	345	155	190	105	35	70	110	20
No. 2		31*	340	290	50	85	60	25	90	50

N, Number of coelomocytes; M, number of labelled coelomocytes; V, number of unlabelled coelomocytes.

*Estimation.

contained ferritin (Fig. 1), showing that they must originate from the somatocoelic epithelium. A piece of pyloric caecum was cut off after each bleeding and its peritoneal labelling was measured (number of iron particles per 300 μm epithelium): there was a progressive decrease of label (Fig. 2). A similar decrease of labelled free cells was found after each successive bleeding (Fig. 1). Thus, the labelled epithelial cells fall progressively into the body cavity.

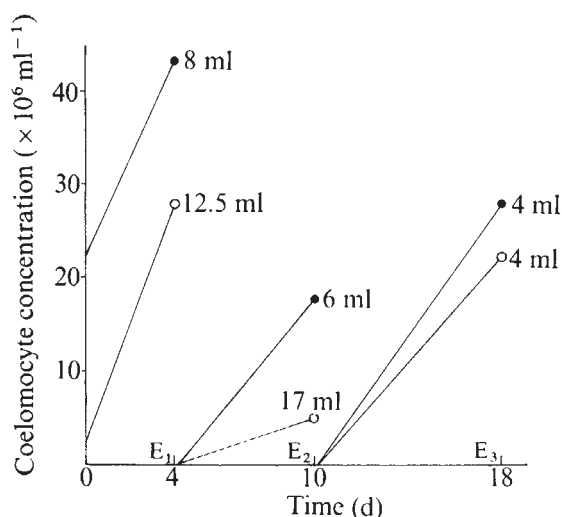


Fig. 3 Change of coelomocyte concentration. Total volume of coelomic fluid is marked for each line.

The introduction of foreign particles into the general coelom induced a considerable increase in the coelomocyte concentration (Fig. 3, T $\rightarrow$ E₁). Similarly, the concentration several days after bleedings E₁ and E₂ shows that the starfish can renew its coelomocytes remarkably quickly.

Table 2 shows the evolution of the total number of coelomocytes in the two starfish we used. Many of the new coelomocytes contained no iron. These cells obviously cannot have come from the division of circulating coelomocytes because the starfish had been bled. Furthermore, the results of injections demonstrate that no cells can have come from outside the coelomic cavity. All this suggests that the new coelomocytes in the general body cavity can have come only from the somatocoelic epithelium. This epithelium thus seems to offer great potential, as it could be the origin of both coelomocytes and digestive epithelial cells (in the case of regeneration)¹⁵.

Observations of *A. rubens* larvae *in vivo* showed that the first free cells appear at the late bipinnaria stage (larvae ± 25 d old). At this stage the subdivision of the coelom is not yet complete and none of the presumed cytopoietic organs is present. Here again, the only possible source of coelomocytes is the coelomic epithelium.

Coelomic epithelial cells have a precisely known structure¹⁶. They are choanocyte-like cells, each apex of which is fitted with a long flagellum surrounded by a crown of micro-

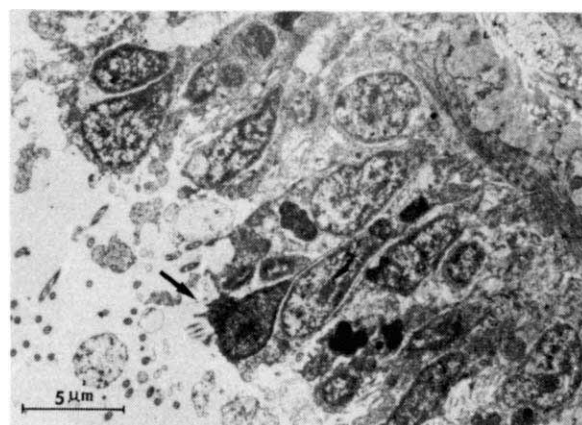


Fig. 4 Transformation of a coelomic epithelial cell into a coelomocyte (epithelium of the rectal caeca).

villi. To form the coelomocytes, the cells detach themselves from the epithelium by stretching from their base (Fig. 4). Shortly after release, each cell loses its flagellum. This happens very fast, and it was by stimulating the formation of coelomocytes by injecting ferritin or Indian ink that we were able to observe it. This explains why flagellated cells had not previously been reported in the coelomic cavities of *A. rubens*¹².

We conclude that the sole origin of coelomocytes in *A. rubens* is the coelomic epithelium and thus all organs lined with such epithelium can be considered to be cytopoietic.

J. P. VANDEN BOSSCHE
M. JANGOUX

Laboratoire de Zoologie,
Collectif de Bio-écologie,
Université libre de Bruxelles,
B-1050 Bruxelles, Belgium

Received February 18; accepted March 18, 1976.

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Electrophoretic evidence that selection reduces ecological overlap in marine limpets

CHARACTER divergence between sympatric, ecologically similar species has been attributed to selection which tends to increase the ecological difference between such species and to reduce the zone of their ecological overlap¹.

Evidence for this phenomenon is based solely on morphological characters which are presumably controlled by a number of genetic factors. There is, however, no documentation of divergence between sympatric species at the allelic level. During the course of a study of the population genetics of the intertidal limpet *Acmaea pelta*, I discovered what seemed to be an effect of coexistence with congeners on the frequencies of allozymes at a leucine aminopeptidase (*Lap*) locus. Populations of this highly eurytopic species from habitats offering widely different algal food and physical conditions, had similar frequencies of *Lap* allozymes, except from habitats where the populations were mixed with large populations of another acmaeid species (unpublished results).

A. pelta is one of fifteen intertidal species of its genus that are sympatric along the rocky shores of central California. A few of these species are rigidly stenotopic obligates of a single plant species, but most are eurytopic species that overlap considerably in their vertical distribution as well as in their utilisation of algal food^{2,3}. A further

study of the *Lap* loci of nine species of *Acmaea* using starch gel electrophoresis showed that each species has one *Lap* locus that gives a gel pattern of either two or four bands per individual. The pattern may be explained by proposing two polymeric enzymes which share a common polymorphic subunit as well as having different monomeric subunits⁴. Thus homozygotes show one band pair and heterozygotes two. This genetic interpretation of the patterns is supported by the fact that in none of the samples did the zygotic frequencies differ significantly from Hardy-Weinberg equilibrium, assuming two Mendelian alleles per animal. A total of eight band pairs were found each of

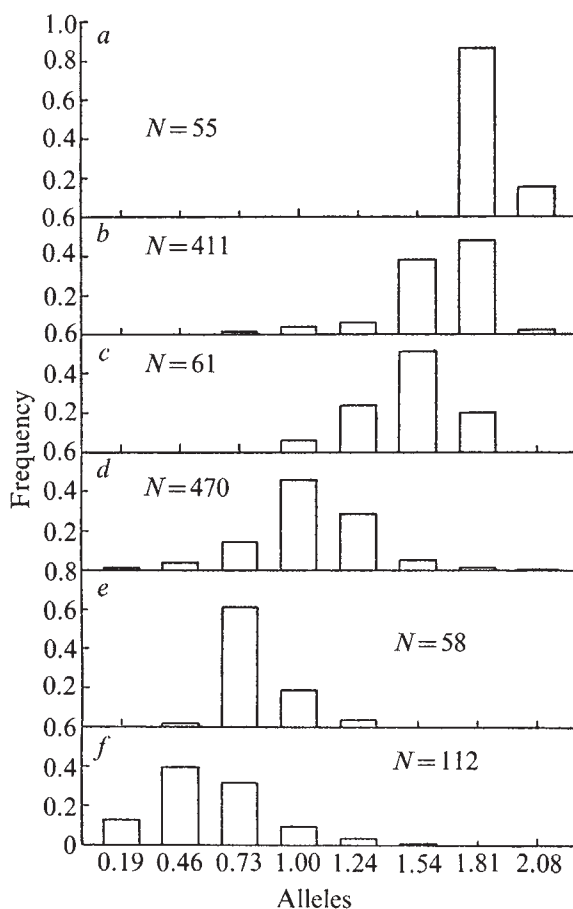


Fig. 1 *Lap* allele frequency distributions of six eurytopic species of the genus *Acmaea*. a, *A. persona*; b, *A. digitalis*; c, *A. scutum*; d, *A. pelta*; e, *A. strigatella*; f, *A. scabra*. Animals were held in running seawater for <24 h after capture. Extracts were made from live tissue, or from animals frozen intact and stored for less than two weeks at -20°C . Repeated electrophoresis of individual extracts shows that storage for over a month at -20°C results in a slight loss of *Lap* activity, but no change in electrophoretic pattern. The hepatopancreas of each limpet was removed and homogenised in an equal volume of gel buffer. After centrifugation, the extract was absorbed with 5-mm squares of Whatman 3MM filter paper. The squares were inserted into Electrostar gels cast in moulds described by Smithies⁵. Gels were prepared and stained according to Brewer⁶, except: 4% (w/v) sucrose was added to the gel; 0.32 M sodium citrate at pH 7.1 was used for electrode buffer, and a constant current of 10 mA was applied for 6 h. This method produced clear zymograms with good resolution of all allelic variants. Internal standardisations of mobility variants were carried out by including *A. pelta* individuals of known genotype on each gel. N, number of animals.

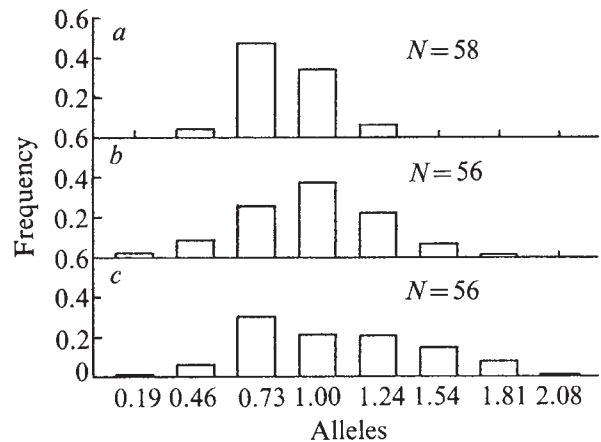


Fig. 2 *Lap* allele frequency distributions for three specialised species of the genus *Acmaea*. a, *A. instabilis*; b, *A. inessa*; c, *A. asmi*.

which corresponded in mobility to one of the pairs found in *A. pelta*. The inferred alleles responsible for the mobility of these band pairs are designated $Lap^{0.19}$, $Lap^{0.46}$, $Lap^{0.73}$, $Lap^{1.00}$, $Lap^{1.24}$, $Lap^{1.54}$, $Lap^{1.81}$ and $Lap^{2.08}$, in order of increasing anodal mobility, with the most frequent allele of *A. pelta* as standard. Each species possesses two or more of these alleles. Figure 1 shows the frequencies of these alleles in six of the eurytopic species that commonly occur in mixed acmaeid assemblages. All of these species have different distributions of *Lap* alleles.

A. pelta is the most eurytopic of these species and coexists, at times, with all species in Fig. 1 except *A. persona*. *A. scutum* often coexists with *A. pelta* in the mid-intertidal zone. Both here and at high intertidal zones *A. scabra* and *A. digitalis* often occur in close association; either of these species can coexist with *A. persona* in the upper reaches of their vertical ranges. Lower down, either can coexist with *A. strigatella*, although *A. digitalis*-*A. strigatella* associations are more common than *A. scabra*-*A. strigatella* associations. The *A. scabra*-*A. digitalis* association has been studied experimentally by Haven⁷ and constitutes the only documented instance of interspecific acmaeid competition.

Since coexistence of ecologically similar species is likely to result in selection that will cause changes at many gene loci, the operation of this selection on closely related species that share identical alleles may have resulted in the species differences shown in Fig. 1. A precedent for the selective importance of the charge on molluscan *Lap* allozymes has been reported by Koehn and Mitton⁸. If the allelic differences among species that commonly coexist are instances of divergence resulting from selection to reduce ecological overlap, then a primitive or optimal distribution of alleles should be preserved in species which, in spite of their sympatry with others, do not compete. Several of the Californian acmaeids should escape competition since, as theorised by MacArthur and Levins⁹, narrowly specialised species do not compete for those resources for which they

are specialised. Three of these species are *A. instabilis*, an epizoite on Laminarian kelps, *A. insessa*, an epizoite on the kelp *Egregia menziesii*, and *A. asmi*, an epizoite on living shells of the snail *Tegula funebris*. The distributions of *Lap* alleles in these three species tend to be broad, and all three have similar central tendencies (Fig. 2).

All eurytopic acmaeids that occur in mixed assemblages are also found in unspecific populations in some habitats. According to my interpretation of the above evidence, selection should operate only in habitats where species coexist, and there the interspecific similarity at the *Lap* loci should be a minimum. In order to test this prediction, habitats along 2 km of coast at Pigeon Point, San Mateo County, California, and along 200 m of coast at Pacific Grove, Monterey County, California, were chosen on the basis of their acmaeid assemblages. At each location samples were taken from habitats that supported unspecific populations of *A. pelta* or *A. digitalis*; samples of both species were also collected from a habitat that supported

mixed populations of *A. pelta* and *A. digitalis*. In addition, at Pigeon Point, a sample of *A. scabra* from a unspecific population, and samples of *A. scabra* and *A. digitalis* from mixed populations, were collected. The results of the *Lap* allozyme analyses of these samples are shown in Fig. 3. In all instances, allelic distributions of coexisting populations differed more than the distributions of unspecific populations of the same species from the same location. The allelic distribution of only one species of each coexisting pair shifted in the predicted direction by a statistically significant ($P < 0.05$) amount using a one-tailed Kolmogorov-Smirnov two-sample test¹⁰. At Pigeon Point, where *A. digitalis* was twice as numerous as *A. pelta* in their shared habitat, the allelic distribution of *A. pelta* was affected (Fig. 3a), but that of *A. digitalis* was displaced at Pacific Grove, where it was mixed with about an equal number of *A. pelta* (Fig. 3c). This agrees with a model of character displacement at one locus proposed by Crozier¹¹, which predicts that competition will cause displacement in both of a pair of sympatric species only in a narrow range of relative reproductive rates, and otherwise displacement will occur only in the more slowly reproducing species.

The selection mechanism responsible for the observed genetic displacement is not known for certain. The possibility of selective settling of planktonic larvae of these limpets cannot be ruled out. Post-larval migration over short distances can be shown to be important in the distribution of *A. digitalis* of different shell patterns to appropriately coloured substrates¹². Migration of settled acmaeids is, however, limited by their inability to cross sandy areas¹³, which would have made post-larval migration between habitats unlikely in most of the cases I studied. Differential survival may be the agent, but my data show no significant correlation between size and genotype. This may mean that selection operates primarily on limpets too small for electrophoretic analysis.

Allelic accommodation in apparent response to competition has not been reported before, and, with acmaeids, should make possible the experimental study of the genetics involved in phenomena such as character displacement and niche diversification.

I thank A. R. Moldenke and J. L. Cox for their suggestions and criticism of an earlier draft of the manuscript. This work was partially supported by the NSF.

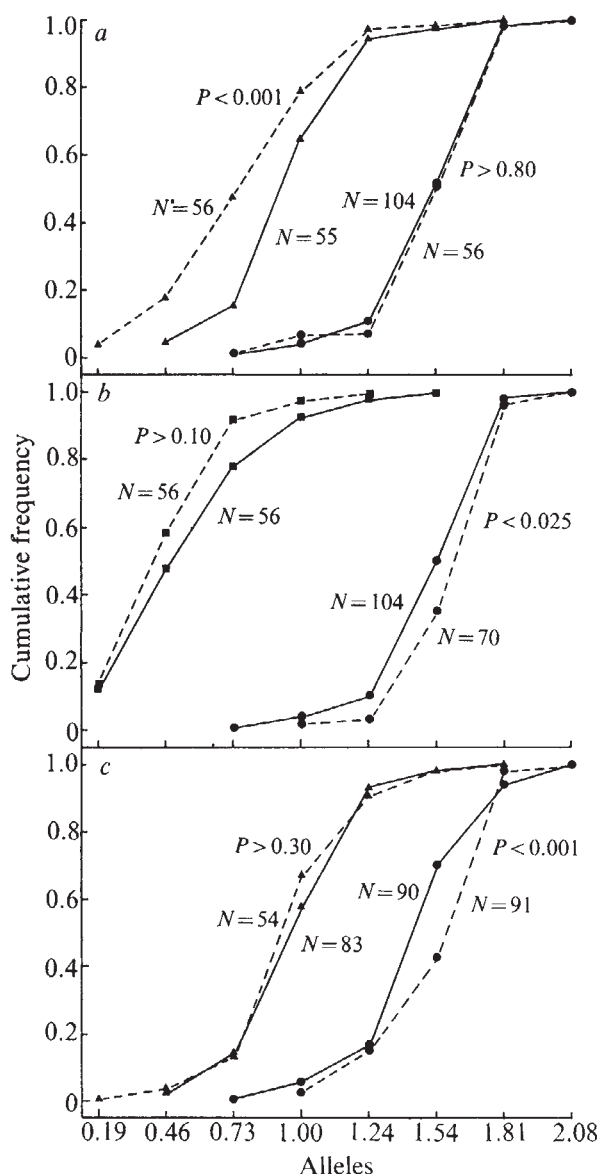
PHILIP G. MURPHY

Hopkins Marine Station,
Pacific Grove,
California 93950

Received February 5; accepted April 1, 1976.

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Fig. 3 Cumulative frequency diagrams of *Lap* alleles of unspecific (—) and coexisting (---) populations of limpets. ▲, *A. pelta*; ●, *A. digitalis*; ■, *A. scabra*. a and b, Pigeon Point, San Mateo County, California; c, Pacific Grove, California. *P* derives from the Kolmogorov-Smirnov test of the H_1 that the allelic distribution of the coexisting population is not shifted in the predicted direction. *N*, number of animals.



Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp.

COROLLA-COLOUR polymorphisms occur in wild populations of many angiosperms, but little is known of the selective factors that maintain them. Valentine has suggested that

yellow/white corolla-colour polymorphisms may be adaptively neutral¹. I have observed extremely strong discrimination by some insect pollinators between the yellow and white corolla-colour forms of polymorphic wild radish. These observations, which are described below, indicate that corolla-colour differences are of adaptive importance in this and similar cases.

I have investigated the behaviour of pollinating insects visiting the flowers of wild radish (*Raphanus raphanistrum* L.), a self-incompatible annual² in which mixed populations of white-flowered and yellow-flowered morphs with sharply different corolla reflectance spectra (Fig. 1) are common in southern Britain. These forms differ only in a single allele, and no other characters have been observed to be associated with the difference in flower colour. Pollinator behaviour was observed in five polymorphic populations in central England and southern Wales during the summer of 1975. Bumble bees (*Bombus* spp.) and sometimes honey bees (*Apis mellifera*) were the chief pollinators, but in all populations *Pieris* spp., especially *P. rapae* (Lepidoptera, Pieridae) were important, often making 10–15% of the total number of visits to *R. raphanistrum*. *Eristalis* spp. (Diptera, Syrphidae) were minor pollinators, comprising up to ~5% of the visitors to *R. raphanistrum*.

Pieris spp. and *Eristalis* spp. showed a strong or very strong preference for the yellow-flowered morph of *R. raphanistrum* in four populations in which the frequencies of the yellow morphs ranged from 7.3 to 60.8% (Table 1). At the Sketty site, for example, where the frequency of the yellow-flowered morph was 60.8%, 307 visits by female *P. rapae* to *R. raphanistrum* were observed; 306 of these visits were to yellow flowers and only one to a white flower. *P. napi* and *P. brassicae* showed an equally strong preference for yellow-flowered *R. raphanistrum* at the same site. At this site, and at Brownhills and Beenham, *R. raphanistrum* was abundant, with yellow-flowered and white-flowered morphs closely intermingled in a mixed stand of field weeds. The Singleton population, which was the only artificial population, consisted of well-spaced rows of F₂ plants segregating at random for yellow or white flower colour. Although the frequency of yellow-flowered plants was only 27.3% in this population, the proportion of visits made to yellow flowers by *P. rapae* was higher than at the Brownhills site (42.3% yellows), and the proportion of visits made to yellow flowers by *E. arbustorum* was higher than at any other site, perhaps as a result of the spatial separation of individual plants at Singleton.

The Landimore population differed from the others both in the low frequency of the yellow morph (0.8%) and in the occurrence of abundant *Sinapis arvensis* L., a crucifer with yellow flowers which resemble those of *R. raphanistrum* and have a closely similar reflectance spectrum to that

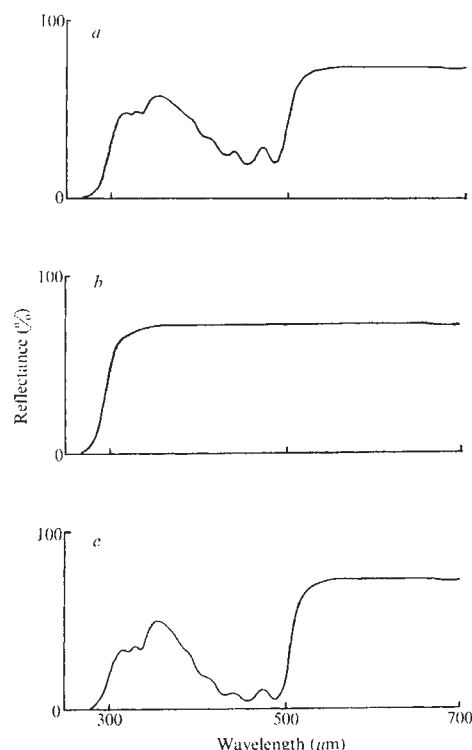


Fig. 1 Reflectance spectra of petals. Measurements were made using a Unicam SP 8000 recording spectrophotometer fitted with an SP 890 diffuse reflectance unit. a, *R. raphanistrum*, yellow morph; b, *R. raphanistrum*, white morph; c, *Sinapis arvensis*.

of yellow *R. raphanistrum* (Fig. 1). *E. arbustorum*, *E. tenax* and other *Eristalis* spp. were abundant on *S. arvensis* flowers at this site, but did not visit *R. raphanistrum*. *P. rapae* and *P. napi* visited white-flowered *R. raphanistrum* and *S. arvensis* with approximately equal frequency. Only two cases of apparently preferential visits to yellow-flowered *R. raphanistrum* were observed at Landimore; a male *P. rapae* was involved in each case. Most male *P. rapae*, and all *P. napi* and female *P. rapae*, did not seek yellow-flowered *R. raphanistrum*.

In all populations the distances that were flown by individuals of the *Pieris* spp. between visited plants were commonly one to several metres, and flights of 15–40 m between successively visited plants were not unusual, with total distances of at least 150 m being covered during a series of visits. Recognition and location of yellow-flowered plants seemed to be entirely visual. Differences in behaviour between different individuals of *P. rapae* were observed at

Table 1 Frequency of visits by pollinators to the yellow-flowered morph of *R. raphanistrum* in five polymorphic populations

Site	Frequency of yellow morphs in population (%)	% of total visits made to yellow flowers					
		<i>P. rapae</i>	<i>P. napi</i>	<i>P. brassicae</i>	<i>E. arbustorum</i>	<i>E. tenax</i>	
		Males	Females				
Sketty	60.8 (418)	94.5† (292)	99.7† (307)	99.1† (111)	100† (107)	89.0† (556)	87.7† (235)
Brownhills	42.3 (130)	89.8† (303)	81.1† (169)	ND	ND	66.0* (47)	70.7† (75)
Singleton	27.4 (73)	92.3† (1,769)	89.3† (131)	93.7† (142)	ND	96.0† (569)	ND
Beenham	7.3 (303)	27.4† (446)	25.8† (190)	ND	ND	ND	40.8† (49)
Landimore	0.8 (240)	4.8† (268)	0.0 (233)	0.0 (470)	ND	ND	ND

The size of each sample is shown in parentheses. Significant differences from the population morph frequencies are indicated: * $P < 0.01$; † $P < 0.001$. The grid references of the sites are as follows: Sketty, SS 623921; Singleton, SS 629917; Brownhills, SK 068052; Beenham, SU 592690; Landimore, SS 473926. ND, not done.

all sites but were most obvious at the Beenham site, where less than half of the individuals of both sexes sought yellow-flowered *R. raphanistrum*. In the natural populations, the strength of the preference that *P. rapae* showed for the yellow morph clearly decreased with decreasing frequency of the yellow morph. Pollinator discrimination of this type may have a role in determining the balance of the flower-colour morphs in *R. raphanistrum*, which shows both geographic clinal variation and local mosaic differentiation in flower-colour morph frequency. In Britain, the white morph predominates in polymorphic populations in southern and eastern England, but the yellow morph predominates or forms monomorphic populations in western and northern Britain.

There have been few investigations of pollinator behaviour in plant populations with flower-colour polymorphism, and no other case has been described in which pollinators discriminated so strongly between flower-colour forms of a wild species. Levin³ found differences in attractiveness of about 1 : 1.49 between the least attractive (coral) and the most attractive (lavender) colour-forms of *Phlox drummondii* Hook. in artificial populations of mixed cultivars; he used pollen-load on the stigma as a measure of attractiveness, but did not observe the behaviour of pollinator species (mainly Lepidoptera) directly. Mogford⁴ observed that some species of *Bombus* discriminated between purple and white flower-colour morphs of *Cirsium palustre* (L.) Scop. in some mixed populations; the greatest discrimination was shown by *B. lapponicus*, which made 88.4% of its visits to the white morph in a population with only 64.0% of white morphs, but discrimination was not consistent. The preference shown by *Pieris* spp. for the yellow morphs seems to conflict with the observations of Ilse⁵ and Bennett⁶, who reported preference for blue, violet, purple and white colours and relative avoidance of yellow flowers of various types by *Pieris* spp. The reflectance spectrum of yellow *R. raphanistrum*, however, shows a complex pattern of reflectance in the near ultraviolet between 275 and 400 nm (Fig. 1) and is thus bee purple⁷ to bees and other insects with trichromatic colour vision extending into the near ultraviolet. Work on the spectral sensitivity of the eye of the butterfly *Heliconius*⁸ has suggested that *P. rapae*, which is certainly sensitive to near ultraviolet reflectance⁹, has this type of colour vision, and similar work on the spectral sensitivity of *E. tenax*¹⁰ suggests that *Eristalis* spp. may have a similar type of colour vision. Ilse and Bennett did not consider ultraviolet colour components. Ilse¹¹ investigated the behaviour of *E. tenax* in relation to flowers of different colours, and successfully trained *E. tenax* to prefer yellow flower models (of Ostwald yellow paper, probably lacking an ultraviolet component) to those of other colours. Obara⁹ observed that male *P. rapae* strongly preferred yellow to white paper models of females in summer, but did not discriminate in autumn; the near ultraviolet component of the colour may have been important. I did not, however, observe any consistent difference between male and female *P. rapae* in preference for the yellow morphs of *R. raphanistrum*, and it seems likely that feeding behaviour rather than mating behaviour determines the flower-colour preferences of *Pieris* spp.

Q. O. N. KAY

Department of Botany and Microbiology,
University College of Swansea,
Singleton Park, Swansea SA2 8PP, UK

Received January 28; accepted March 29, 1976.

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Vegetative reproduction and close packing in a successional plant species

HERBACEOUS perennials with determinate shoots commonly produce new aerial stems by rhizomes, stolons or adventitious root buds^{1–3}. The usual result is a more or less dense clone. Preliminary field studies were made of tightly packed clones of goldenrods (*Solidago* spp., Compositae), common in late herbaceous stages of succession in the eastern USA. These studies suggested orderly geometric patterns of rhizome production.

A geometric model for development of dense clones was devised (Fig. 1). It was assumed that an optimal pattern of stem production maximises rate of centrifugal spread, maintains sufficient stem density in already colonised sites to produce a closed canopy, and minimises total rhizome length (and thus, biomass and respiratory load). The model requires that most parental rhizomes produce two or more daughter rhizomes, and that most daughter rhizomes diverge from the direction of parental growth by 60°, with one growing to the left (–60°) and one to the right (+60°) of the parental line of growth. Such a pattern, by itself, produces frequent gaps in the clone. The model also requires that occasional daughter rhizomes continue in the direction of parental growth. The stems subsequently produced by these 'non-divergent' rhizomes reduce the number of gaps left by divergent (60°) rhizomes. They also increase the rate of clone expansion.

The model incorporates some redundancy—that is, in ideal conditions, more than one stem may be produced at

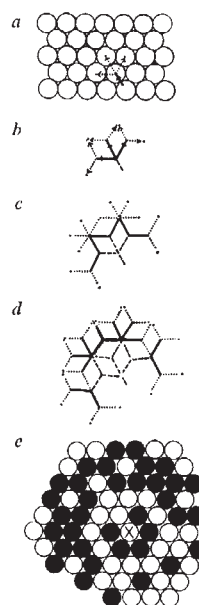


Fig. 1 Model of rhizome development during a 5-yr sequence. Dotted lines represent new rhizomes terminating in live aerial stems; solid lines represent 1-yr-old rhizomes; dashed lines represent rhizomes 2 yr old or more; a, parent rhizome and three daughter rhizomes; b, same system 1 yr later; non-divergent (0°) daughter rhizomes are not produced in this and subsequent years; c, same system 2 yr later; d, same system 3 yr later; e, 4 yr later; ●, represents a space filled by foliage from one or more aerial stems; gaps could be filled by more frequent production of non-divergent rhizomes and by production of aerial stems directly from old stem bases; X, point of origin of the three daughter rhizomes shown in 1a.

a given point within the clone. In field conditions, rhizome mortality or damage, combined with environmental heterogeneity, would degrade optimal developmental patterns. Redundancy increases the probability that a given clone will maintain a closed canopy and maintain a rapid rate of centrifugal spread, in spite of environmental variables and developmental errors.

Clones of *Solidago canadensis* L. were used to test the model. *S. canadensis* is a common successional species which can form dense circular clones at least 2 m in diameter. Vegetative reproduction is by rhizomes, which produce aerial stems at their tips. Occasionally, new stems are produced directly from old stem bases. New rhizomes are produced only from stem bases. The study site was a field in eastern Pennsylvania (Lima, Delaware County) which was last ploughed 7 yr before the study. Slope was approximately 6–8% and the soil was a well-drained Glenelg channery silt loam⁶. Fifteen sod blocks were excavated from dense *S. canadensis* clones; each block was 0.7 m long, 0.4 m wide and 15–20 cm deep. Soil was washed off and rhizome geometry measured. Data included: the number of daughter rhizomes produced by each stem base; the rhizome length; and the angle formed by the base of each daughter rhizome and the straight line projected from the end of the parent rhizome. Rhizomes grew horizontally with little variation in depth below the surface. Thus, systems were described in a two-dimensional plane. All rhizomes had scale leaves oriented in the direction of growth, permitting separation of parent and daughter rhizomes.

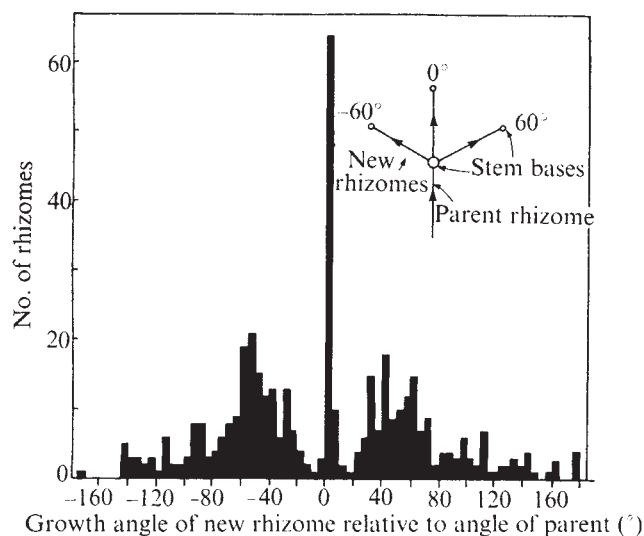


Fig. 2 Frequency distribution of rhizome growth angles. See text for explanation.

Eighteen per cent of all rhizomes excavated continued in the direction of parental growth ('non-divergent'; Fig. 2). Any angle falling within 10° of parental growth direction was considered non-divergent. Of the remaining, divergent rhizomes, those that diverged to the left had a mean angle of $-66.0 \pm 2.41^\circ$ (mean $\pm$ s.e.); those that diverged to the right had a mean angle of $67.6 \pm 2.95^\circ$. Mean number of daughter rhizomes per stem base was 2.43 ± 0.104 .

The data of Fig. 2 do not, in themselves, provide an adequate test of the model. Similar data could result from production of rhizomes diverging only to the right by one-half of all stem bases, and only to the left by the remaining stem bases. This pattern would not result in the predicted clone geometry of Fig. 1. Of the 106 stem bases that produced at least two diverging daughter rhizomes, 85.8% produced at least one rhizome diverging to the left and one diverging to the right. Of the 53 stem bases which produced

at least two diverging rhizomes falling within 1 s.d. of one or both of the mean divergence angles ($-66.0 \pm 32.98^\circ$ and $+67.6 \pm 37.97^\circ$), all had rhizomes falling within 1 s.d. of both left and right means. Thus, it was rare for all rhizomes produced at a given stem base to diverge in one direction.

Rhizome length could not be accurately determined for most systems because of breakage, because older rhizomes decay rapidly, and because the youngest rhizomes had not yet terminated in aerial stems. Mean linear distance between each stem base and the nearest adjacent stem base was 7.5 ± 0.61 cm ($n=170$). It seems that rhizome length is frequently greater than this. New aerial stems are occasionally produced directly from old stem bases, increasing centripetal density. Some rhizomes grow at angles much greater than 60° , further increasing stem density in older portions of the clone.

Mean rhizome angles correspond closely to the model, suggesting some selection pressure for geometrical order. There is considerable variability in growth angle and rhizome frequency, resulting in breakdown of clone symmetry. The high degree of redundancy in the system, however, seems to compensate for this variability, resulting in the commonly observed pattern of closely packed aerial stems.

We thank Mr Franklin Haas, Superintendent of Ridley Creek State Park, for permission to carry out the study; and Drs Jack McCormick and Marilyn Jordan Buchauer for guidance and collaboration during preliminary studies.

ALAN P. SMITH

Department of Biology,
University of Pennsylvania,
and Smithsonian Tropical Research Institute,
Balboa, Canal Zone

JAMES O. PALMER

Department of Biology,
University of Pennsylvania,
Philadelphia, Pennsylvania

Received January 16; accepted March 15, 1976.

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Complementation of *ts* mutants by a herpes simplex virus *ts*-transformed cell line

THE first evidence that human herpesvirus could transform cultured cells was provided by Munyon *et al.*¹ who showed that the thymidine kinase activity of herpes simplex could be expressed in mouse-derived L cells lacking this enzyme (LTK⁻ cells). The oncogenic potential of human herpes virus was demonstrated by Duff and Rapp^{2,3} who showed that hamster embryo cells transformed by ultraviolet-inactivated herpes simplex virus (HSV) were oncogenic in newborn hamsters. Further evidence of HSV transformation has been described in cultured human, rat and mouse cells^{4–7}. The exact role played by HSV in transformation of primary embryo cells and the amount of information (if any) required to maintain the transformed state is not known. Temperature-sensitive (*ts*) mutants of HSV-2, HG 52 have been shown to complement each other⁸. These *ts* mutants may be used in complementation tests together with rat embryo cells transformed by another *ts* mutant to demonstrate the expression of herpes information in the transformed line. We report here evidence of such complementation between an HSV-2 *ts* mutant-transformed rat embryo line and three superinfecting HSV-2 *ts* mutants. This complementation suggests that this oncogenically

Table 1 Yield of virus from transformed and control cells infected with three complementing *ts* mutants of HSV-2

Mutant	No. of PFU in yield* after infection of cells for 24 h at 38.5 °C	
	Control rat embryo cells	RE 1-transformed cells
<i>ts</i> 10	9	432
<i>ts</i> 10	2	298
<i>ts</i> 10	0	14
<i>ts</i> 3	3	210
<i>ts</i> 3	45	416
<i>ts</i> 3	272	578
<i>ts</i> 4	10	300†
<i>ts</i> 4	0	4†
<i>ts</i> 4	2	30†
<i>ts</i> 1‡	0	0
<i>ts</i> 1	2	4
<i>ts</i> 1	404	309
<i>ts</i> 1	0	0
<i>ts</i> 1	0	0

*Plaques obtained on titration at 31 °C: no plaques were found on titration at 38.5 °C.

†Plaques obtained by infection of RE 1 cells by *ts* 4 were consistently larger than those on control plates.

‡Infection of transformed and control cells by *ts* 1, the mutant used for the original transformation of RE 1, was included as a control of the specificity of the complementation.

transformed line can express herpes information sufficient to complement at least three genes.

The transformation of rat embryo cells by *ts* mutants of HSV has been described^{9,10}. Lines of HSV-transformed cells produced by picking foci and passaging these cells in tissue culture, have been shown to express HSV information in immunofluorescence tests with herpes-specific antiserum. Some of the transformed lines have been shown to be tumorigenic in rats (ref. 10 and our unpublished data) and the tumour cells, cultured *in vitro* express HSV information as seen by immunofluorescence. Extracts of tumours and of transformed cells express herpes-specific thymidine kinase (our unpublished data). Serum from tumour-bearing animals contains neutralising antibody and also antibody to herpes-coded thymidine kinase activity. Kimura *et al.*¹¹ have reported that at 38 °C the growth of two *ts* mutants was significantly enhanced in cells transformed by ultra-violet-inactivated HSV-2.

The line of transformed cells used was the RE 1 line described before^{9,10}. Control cells were hooded Lister rat embryo primary cells and "hood" cell lines derived from them. Unpublished experiments have shown that it is not possible to distinguish the RE 1 line from primary rat embryo cells or hood cells by growth curves or saturation density attained. BHK 21 cells were used to prepare stocks of mutant virus and also for titration of virus in the complementation tests.

Tests for complementation were carried out by comparing the yields of virus from transformed RE 1 and from rat embryo control cells. Monolayers of transformed cells and of rat embryo control cells were infected with a *ts* mutant at a multiplicity of infection of 5 PFU per cell. Duplicate plates were used throughout. After adsorption for 1 h at 37 °C the virus inoculum was removed, and the plates were washed twice with Eagle's medium, incubated for 30 min at 38.5 °C with 5% antiserum, washed twice again with Eagle's medium and then incubated in Eagle's medium with 5% calf serum at 38.5 °C for 24 h. The cells were then scraped into the medium, disrupted by sonication and 0.2-ml samples were shaken with a suspension of 6×10⁶ BHK 21 cells for 45 min at 37 °C. These cells were then divided between two 50-mm Petri dishes containing Eagle's medium with 5% calf serum; one plate was incubated at 38.5 °C for 2 d and the other at 31 °C for 3 d. Plates were then stained with Giemsa stain and the number of plaques obtained from infection of the RE 1 cells by a *ts* mutant was compared with the number obtained from

control cells infected with the same mutant. A yield from the RE 1 cells of more than twice the number of plaques obtained from the control cells was regarded as evidence of complementation of the mutant virus by the transformed cells.

Results obtained with the HSV-2 mutants, *ts* 3 *ts* 4 and *ts* 10 are shown in Table 1. In each case there was evidence of complementation of the mutants by the transformed cell line. We have shown by fluorescence that HSV-specific proteins are produced in rat embryo cells infected with *ts* mutants (unpublished data) at 38 °C. This indicates that neither uptake nor uncoating are blocked.

Yields from the transformed cells infected with *ts* 4 regularly produced larger plaques than the parallel yields of control cells infected with this mutant, a finding which is under investigation.

No significant increase in yield was seen when the transformed cells were infected with *ts* 1, the mutant originally used to transform the cells. The information expressed in RE 1 cells cannot complement the homologous *ts* 1 mutant. It is also implied that complementation was specific for the expression of HSV information in the transformed cell line. In addition, the mutants *ts* 2 *ts* 5, *ts* 6, *ts* 7, *ts* 8 and *ts* 9 were tested, in duplicate on at least three separate occasions, but none showed significant complementation (Table 2), indicating that the relevant genetic information to complement these mutants is either absent or not expressed in the RE 1 cells.

Virus from plates showing complementation was tested for the presence of recombinants by replating the yield at 38.5 °C, but no recombinants were detected. Control hood cells after 21 and 34 passages behaved like primary rat embryo cells when infected with *ts* mutants. Thus the complementation of HSV *ts* mutants by RE 1 cells is unlikely to be just a consequence of their multiple passages in culture.

The four mutants *ts* 1, 3, 4 and 10 complement each other in standard genetic complementation tests* and the mutations are therefore in different genes. The fact that cells transformed by *ts* 1 complement the three mutants, *ts* 3, 4 and 10, suggests that at least three HSV functions are expressed in the transformed cells. HSV-specified thymidine kinase activity is expressed in RE 1 cells (our

Table 2 Yield of virus from transformed and control cells infected with six non-complementing *ts* mutants of HSV-2

Mutant	No. of PFU in yield* after infection of cells for 24 h at 38.5 °C	
	Control rat embryo cells	RE 1-transformed cells
<i>ts</i> 7	0	0
<i>ts</i> 7	0	0
<i>ts</i> 7	0	0
<i>ts</i> 5	0	0
<i>ts</i> 5	0	0
<i>ts</i> 5	0	0
<i>ts</i> 9	170	24†
<i>ts</i> 9	0	0
<i>ts</i> 9	0	0
<i>ts</i> 8	0	0
<i>ts</i> 8	0	0
<i>ts</i> 8‡	20	38
<i>ts</i> 2	0	0
<i>ts</i> 2	0	0
<i>ts</i> 2	0	0
<i>ts</i> 6‡	30	50
<i>ts</i> 6	0	0
<i>ts</i> 6	0	0

*Plaques obtained on titration at 31 °C: no plaques were found on titration at 38.5 °C.

†The very small plaques noted here have been seen in a similar experiment done previously.

‡Mutants 6 and 8 do grow somewhat better in RE 1-transformed cells but the results were not significant in this set of data.

unpublished data), but this TK activity is not likely to be relevant to the *ts* mutant complementation demonstrated here, both because it is not normally an essential function for HSV replication¹², and because it is produced in normal amounts by all four mutants at 38 °C (ref. 13). Both *ts* 3 and *ts* 4 are DNA-synthesis-positive mutants but *ts* 1 and *ts* 10 are DNA synthesis negative¹⁴. *ts* 10 is slightly defective in production of viral DNA polymerase at 38 °C whereas *ts* 1 produces somewhat larger amounts than wild-type virus at 38 °C (ref. 13). Polymerase production, however, is probably not the primary defect of *ts* 10 and it is not yet possible to identify the functions involved in the complementation reaction in transformed cells. Our work suggests that the transformed RE1 cells express not only HSV-specific antigens detectable by fluorescence and HSV-specific thymidine kinase activity, but also the HSV genetic information defective in three different HSV *ts* mutants. Our results with seven other mutants show that a large proportion of HSV-specified information is not expressed in these cells.

We thank Professor J. H. Subak-Sharpe for his interest, and Mrs Alice Birrell and Mrs Linda Calder for technical assistance.

JOAN C. M. MACNAB
MORAG C. TIMBURY

MRC Virology Unit and
University of Glasgow,
Institute of Virology,
Church Street,
Glasgow G11 5JR, UK

Received February 16; accepted April 8, 1976.

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Lectin-mediated attachment of glycoprotein-bearing liposomes to cells

LIPOSOMES have been used as vehicles for transporting drugs or enzymes into cultured cells and into tissues¹⁻⁸. Before the full potential of liposome delivery systems can be realised, however, means must be found to direct the drug or enzyme bearing particles to specific sites within the body. Some modulation of the *in vivo* distribution of liposomes can be achieved by altering the size or charge of the vesicles^{9,10}. More precise control of the interaction of liposomes with cells, and their distribution *in vivo*, may, however, be possible through the introduction of specific macromolecular entities into the liposome membrane. As a preliminary step in this direction we have incorporated a macromolecular lectin receptor into liposome membranes and have examined the lectin-mediated attachment of such liposomes to cells *in vitro*.

Our choice as a lectin receptor was the major sialoglycoprotein of the human erythrocyte membrane¹¹. This component was extracted and purified to homogeneity¹² as evidenced by polyacrylamide gel electrophoresis (Coomassie blue staining). The erythrocyte sialoglycoprotein bears

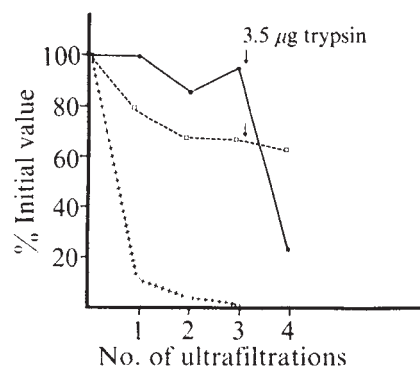


Fig. 1 Sialoglycoprotein liposomes were prepared by injecting 200 µl of an ethanolic solution of lipids (6 µmol egg phosphatidyl choline, 3 µmol cholesterol and a tracer amount of ³H-dipalmitoyl phosphatidyl choline) into 4 ml of Dulbecco's phosphate buffer (pH 7.2). The buffer contained 100-500 µg of erythrocyte sialoglycoprotein and tracer amounts of ¹⁴C-sialoglycoprotein; occasionally, low molecular weight solutes were included for trapping in the aqueous compartment of the liposomes. Free sialoglycoprotein and low molecular weight solutes were separated from liposome-bound material by repetitive ultrafiltration in Diaflow apparatus using an XM-100A membrane. Samples were repeatedly concentrated approximately tenfold by ultrafiltration and then diluted to the original volume. Aliquots were taken and sampled for radioactivity. At the indicated point the sialoglycoprotein liposomes were incubated with 3.5 µg ml⁻¹ twice-crystallised trypsin for 30 min at 37 °C. ○, Free ¹⁴C-sialoglycoprotein (SP); ●, liposome-bound sialoglycoprotein (SP); □, lipid-³H-phosphatidylcholine (PC). The loss of ³H-lipid during the first two ultrafiltrations is probably due to absorption of isotope on to hydrophobic sites in the membrane filter. It can be reduced by prewashing the filter with an albumin solution.

receptor activity for the lectins from wheat germ (WGA) and from *Phaseolus vulgaris* (PHA), but does not have appreciable receptor activity for concanavalin A (con A)^{12,13}. The sialoglycoprotein was incorporated into liposomes which were mainly of the unilamellar type as formed by the technique of Batzri and Korn¹⁴. Briefly, an ethanolic solution of egg phosphatidyl choline and cholesterol (2:1 molar ratio) was injected rapidly into a solution of sialoglycoprotein in phosphate buffer. The sialoglycoprotein-containing liposomes formed were separated from free sialoglycoprotein by ultrafiltration through an Amicon XM 100A membrane, or by filtration on Sepharose 4B. The

Table 1 Haptene inhibition of sialoglycoprotein liposome attachment to red cells

	Expt	WGA(%)	PHA (%)	RCA (%)
Lectin	I	100	100	100
	II	100	100	100
Lectin + haptene	I	47	91	0
Sugar	II	0	0	—
Lectin + non-haptene	I	106	107	125
Sugar	II	—	53	—
Lectin + post-treatment	I	105	—	0
W. haptene	II	99	25	—

Erythrocytes were treated with the indicated concentration of lectins washed and exposed to sialoglycoprotein liposomes for 2 h at 25 °C. In some cases appropriate haptene sugars (*N*-acetylglucosamine for WGA, *N*-acetylgalactosamine for PHA and galactose for RCA) or mannose as a non-haptene sugar were included in the reaction mixture. The samples were then processed as described in Fig. 2. Nonspecific attachment (in the absence of lectin pretreatment) was subtracted from the data. The results are expressed as the percentage of the effect produced by the lectin alone. The results represent the means of triplicate determinations. Experiment I, lectin concentrations were 50, 20 and 80 µg ml⁻¹ for WGA, PHA and RCA, respectively. The haptene and non-haptene sugars were present at a final concentration of 50 mM. Experiment II, lectin concentrations were 100 and 20 µg ml⁻¹ for WGA and PHA, respectively. The haptene and non-haptene sugars were present at a final concentration of 250 mM.

sialoglycoprotein from erythrocytes has previously been incorporated into artificial lipid membranes by several other workers¹⁵⁻¹⁸.

The degree of incorporation and the ratio of protein to lipid in the liposome preparations were measured using ³H-dipalmitoyl phosphatidyl choline and ¹⁴C-sialoglycoprotein (labelled by reductive alkylation with ¹⁴C-formaldehyde¹⁹), both of known specific activity. The sialoglycoprotein was readily incorporated into the liposomes at low protein-lipid ratios and somewhat less efficiently incorporated at higher ratios. Thus the percentage incorporation varied from 90 to 30% using 10–200 µg protein per mg phosphatidyl choline. Most of our preparations had a sialoglycoprotein-phosphatidyl choline weight ratio of 0.10–0.30, but higher ratios could also be achieved. Assuming a mean molecular weight 2×10^6 for unilamellar vesicles²⁰, and a molecular weight of 53,000 (ref. 12) for the sialoglycoprotein, then most of our preparations contained an average of 2–6 sialoglycoprotein molecules per vesicle. Much of the incorporated sialoglycoprotein seemed to be arrayed at the outer surface of the liposome since it could be cleaved from the vesicles by low doses of trypsin (Fig. 1a). In experiments using 3.5–75.0 µg ml⁻¹ trypsin, from 72 to 87% of the sialoglycoprotein was cleaved from the vesicles as judged by release of radioisotope. It is unlikely that the protease penetrated into the vesicles in the conditions used since incubation with substantially higher doses (100 µg ml⁻¹) of trypsin failed to release any of the

³H-sucrose sequestered within the aqueous compartment of the liposomes. These results contrast with those of MacDonald and MacDonald¹⁷ who used another technique for preparing sialoglycoprotein liposomes and found only 50% of the incorporated protein exposed at the outer surface of the vesicles. We have not yet characterised the physical properties of the sialoglycoprotein liposomes in great detail; however, columns of Sepharose 4B largely retarded these liposomes yielding an elution profile typical of a preparation containing mainly small unilamellar vesicles²¹.

Liposomes bearing sialoglycoproteins can participate in agglutination reactions mediated by appropriate lectins such as WGA. This can be in the form of self agglutination as first described by Redwood and Jansons (ref. 16 and Fig. 2a) or it can be mixed agglutination with lectin-coated cells such as erythrocytes (Fig. 2b). Both types of agglutination reaction exhibit apparent saturation and display a sigmoidal dependence on the amount of lectin used. The rate of lectin-mediated attachment of liposomes to cells is rapid at first and then slows substantially, with maximal attachment after 3–4 h at 25 °C (Fig. 2c). The mixed liposome-cell agglutination reaction seems slow compared with the reported kinetics of lectin-mediated agglutination of cells, where the reactions went to completion in about 20 min (ref. 22). The participation of the liposomes in lectin-mediated agglutination to cells is governed by the receptor specificities borne by the sialoglycoprotein. Thus WGA and especially PHA, which have high affinities for isolated sialoglycoprotein¹²,

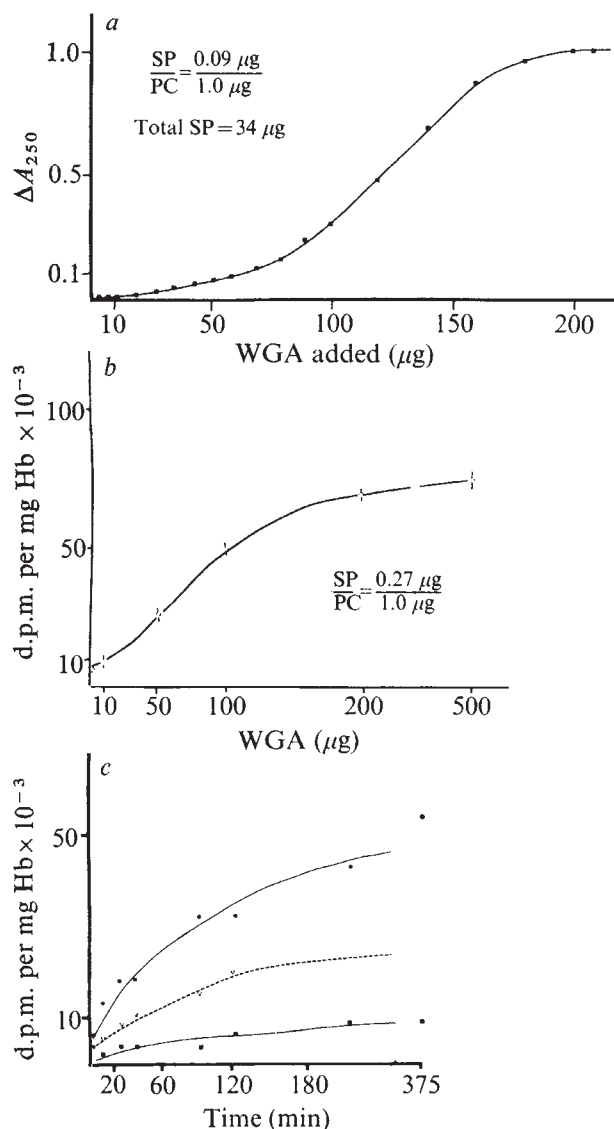


Fig. 2 a, Self agglutination of sialoglycoprotein liposomes. The agglutination of sialoglycoprotein liposomes was followed by light scattering measurements in a Beckman CII double-beam spectrophotometer. The absorbance of a sample cuvette containing sialoglycoprotein liposomes and WGA was compared with that of a reference cuvette containing an equal amount of sialoglycoprotein liposomes and WGA as well as 100 mM *N*-acetyl glucosamine. The difference in absorbance A_{250} was determined just after stirring both cuvettes and 5 min after each addition of WGA. The sialoglycoprotein (SP)–phosphatidyl choline (PC) ratio is indicated. Attachment of sialoglycoprotein liposomes to cells—experiments involving the binding of sialoglycoprotein liposomes to erythrocytes were conducted as follows. Aliquots (5 ml) of a 2% suspension of human red cells in Dulbecco's buffer were incubated with the indicated doses of lectins for 30 min at 4 °C. The lectin-coated cells were recovered by centrifugation and washed three times in buffer. Clumps of cells were dispersed by gentle pipetting. Aliquots (2 ml) of lectin-coated red cells in buffer were incubated with 100 μl of sialoglycoprotein liposomes containing ³H-dipalmitoyl phosphatidyl choline at 25 °C and usually for 120 min with constant mixing. The liposome-cell mixture was layered on 2 ml of a 10% albumin solution in buffer and the red cells were separated from unbound liposomes by sedimentation at 2,500 r.p.m. for 15 min. The cell pellet was dispersed and aliquots were taken for haemoglobin determination and for radiation counting. Efficiency corrections were made using a curve generated from samples quenched with whole blood. **b**, Dose dependence of WGA-mediated attachment of sialoglycoprotein liposomes to red cells at 25 °C. Abscissa, WGA (μg) used in pretreating red cells; ordinate, d.p.m. of ³H-phosphatidyl choline per mg of haemoglobin. Mean $\pm$ s.e. shown ($n=3$). **c**, Kinetics of WGA-mediated attachment of sialoglycoprotein liposomes to red cells. Erythrocytes were treated with 100 μg of WGA in buffer, or with buffer only. The time course of attachment of sialoglycoprotein liposomes to cells at 25 °C was measured as above. ●, + WGA; ▽, + WGA + 50 mM *N*-acetylglucosamine; ■, – WGA. For these experiments wheat germ agglutinin (WGA) and con A were obtained from Sigma, crude PHA was from Difco. RCA was prepared as described previously³⁰.

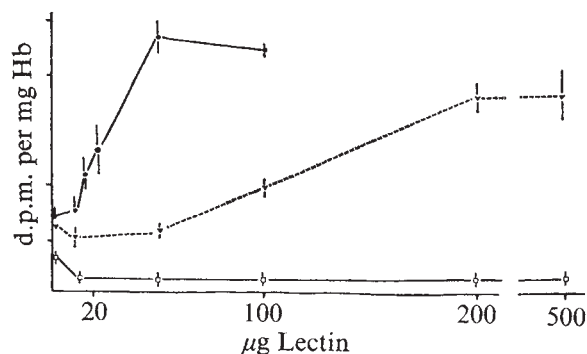


Fig. 3 Dose dependence of lectin-mediated attachment of sialoglycoprotein to erythrocytes at 25 °C. Protocol as in Fig 2b. Abscissa, lectin (μg) used in pretreatment of erythrocytes; ordinate, d.p.m. of ^3H -phosphatidyl choline per μg of haemoglobin. $\bullet$, PHA; $\blacktriangledown$, RCA; $\square$, con A. Mean $\pm$ s.e. shown ($n=4$).

are effective in promoting liposome attachment to cells, whereas con A, which does not interact with the sialoglycoprotein, fails to cause liposome-cell attachment (Fig. 3). A possible anomaly in our results seems to be the case of *Ricinus communis* agglutinin (RCA) which has been reported to interact only weakly with the isolated sialoglycoprotein¹², but which promotes strongly sialoglycoprotein liposome attachment to cells (Fig. 3). This discrepancy may be due to the presence of RCA receptor components in our sialoglycoprotein preparation which react poorly with Coomassie blue or with periodic acid-Schiff stains. RCA receptors from erythrocytes are known to have such characteristics and to overlap partially with the major sialoglycoprotein in gel electrophoresis¹³. As Table 1 shows, the presence of appropriate hapten sugars reduces the lectin-mediated attachment of sialoglycoprotein liposomes to cells. Only in the case of RCA, however, can the liposomes be fully released from the cell-liposome aggregate by post-treatment with the hapten sugar. It seems likely, in this case at least, that the liposomes are bound at the surface of the erythrocyte and are not internalised.

The maximal levels of lectin-mediated sialoglycoprotein liposome attachment to erythrocytes seen in Figs 2 and 3 represent the binding of 10–25 μg of phospholipid per mg of haemoglobin. By comparison, the normal human erythrocyte contains approximately 8.9 μg of phospholipid per mg of haemoglobin²³; thus the liposomes bound to the erythrocytes by lectins comprise 2–3 times the total lipid of the red cell membrane. Since these liposomes probably possess a single bilayer membrane, our observations would suggest that, at maximal levels of attachment, the erythrocyte surface is largely covered by the bound vesicles.

Table 2 Efflux rates of $^{35}\text{SO}_4^{2-}$ from liposomes

	First hour	Second hour	Third hour
Sialoglycoprotein liposomes	11.8	11.4	9.8
Sialoglycoprotein liposomes + 100 $\mu\text{g ml}^{-1}$ RCA	16.2	14.0	13.9
Sialoglycoprotein liposomes + 100 $\mu\text{g ml}^{-1}$ RCA + 50 mM galactose	12.5	10.8	8.4
Sialoglycoprotein liposomes + 1 mg ml^{-1} RCA	26.6	18.0	12.9
Sialoglycoprotein liposomes + 1 mg ml^{-1} RCA + 50 mM galactose	16.5	13.8	11.8
Triton X-100 (0.5%)	77.3		

Sialoglycoprotein liposomes were prepared containing trapped $\text{Na}_2^{35}\text{SO}_4$ and the efflux rate at 25 °C of $^{35}\text{SO}_4^{2-}$ was measured as previously described²⁰. Results are expressed as percentage efflux per h.

To assess the possibility that lectins disrupt the structure of sialoglycoprotein liposomes during aggregation reactions, we have studied the efflux of $^{35}\text{SO}_4^{2-}$ from such liposomes. Table 2 shows that the efflux rate of $^{35}\text{SO}_4^{2-}$ from sialoglycoprotein liposomes was approximately 10% per h, which is within the range commonly observed for anion fluxes in negatively charged liposomes²¹. The introduction of a lectin (RCA) at a concentration up to 1 mg ml^{-1} had less than a twofold effect on the efflux rate, and some of this effect may have been due to nonspecific protein-lipid interaction. These results suggest that lectin-mediated aggregation of sialoglycoprotein liposomes does not result in gross perturbation of the structure of the liposome membrane.

The SP liposome-lectin system described here may be applicable to investigation of the biochemical basis of lectin-mediated agglutination of cells. This phenomenon is a complex one, involving the number and affinity of lectin receptors, the mobility of receptors in the membrane and the interactions of receptors with active cytoplasmic elements^{22,24,25}. In our system, several of these factors are subject to precise experimental manipulation. For example, the average receptor density per particle may be varied by altering the lipid-protein ratio during liposome formation. The receptor mobility may be controlled by preparing liposomes from synthetic phospholipids with known transition temperatures, followed by investigation of the agglutination reaction above or below the thermal transition. This approach may be complementary to studies using glutaraldehyde fixation to modify receptor mobility²⁶ since fixation may indirectly affect receptor mobility by altering intracellular elements such as microtubules or microfilaments, whereas alteration of the lipid composition of the SP liposome would directly modify membrane fluidity.

The system we have studied, namely the mixed aggregation of receptor-bearing liposomes and cells, may have some advantages over systems which use the self aggregation of liposomes^{16,27}. Thus, we can examine the interaction of a well characterised receptor-bearing particle with undefined receptors on cells. The effects of manipulating cellular physiology on the aggregation reaction can also be studied.

We feel that our studies represent a step towards the development of liposomes which can be 'targeted' to specific cell types *in vitro* or *in vivo*. During the course of these studies Gregoriadis and Neerunjun²⁸ reported on the use of specific antibodies to 'target' drug bearing liposomes *in vitro*. Our endeavours and those from Gregoriadis' laboratory seem similar in intent although different in approach.

This work was supported by the National Cancer Institute of Canada. We thank Dr D. Papahadjopoulos for ^3H -dipalmitoyl phosphatidyl choline.

R. L. JULIANO*
D. STAMP

Research Institute,
The Hospital for Sick Children,
555 University Avenue,
Toronto, Ontario, Canada

Received January 30; accepted March 24, 1976.

*Also at: Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Ontario.

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Mycoplasma pneumoniae is a polyclonal B-cell activator

MYCOPLASMA PNEUMONIAE (MP) is an important causative agent of lower respiratory tract illness in man¹. Lymphocytes from subjects who have experienced MP infection show a high degree of stimulation when cultured in the presence of MP antigen^{2,3}. But high concentrations of MP antigen also stimulate, to some extent, lymphocytes from individuals without serological evidence of previous MP infection, suggesting that these subjects have either been infected with MP in the past or that MP also has a non-antigen-specific, mitogenic effect². Several mitogens are known: the plant lectins phytohaemagglutinin (PHA) and concanavalin A, for example, have a mitogenic effect on T lymphocytes⁴, and certain bacterial products, such as lipopolysaccharide (LPS) of *E. coli*⁵ and a purified protein derivative of tuberculin (PPD) are mitogenic for mouse B cells⁶ and induce polyclonal antibody synthesis in mouse spleen cells⁴. These substances are called polyclonal B-cell activators (PBA) (ref. 6). We now report that MP is mitogenic for mouse and guinea pig lymphocytes and induces polyclonal antibody formation in mouse spleen cells.

Cells were obtained from inbred C57BL mice, specific pathogen-free congenitally athymic Nu/Nu mice on a

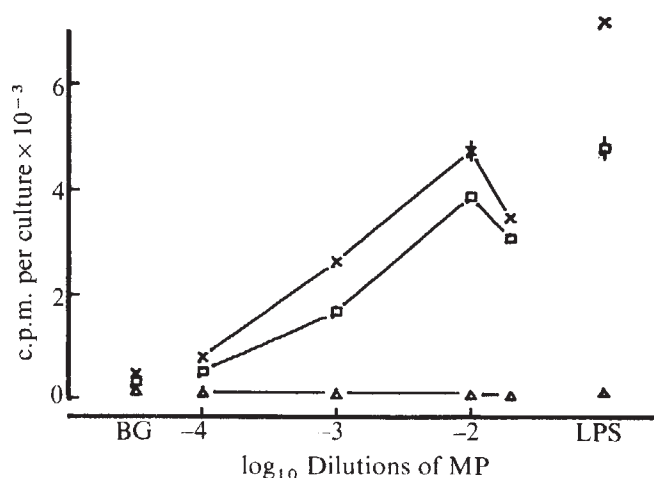


Fig. 1 Spleen cells from 6-week-old C57BL mice were cultured in the presence of MP (*Mycoplasma pneumoniae*) or LPS (lipopolysaccharide) (100 µg) at a cell concentration of 5×10^6 cells per culture in serum-free medium. DNA synthesis was measured after 2 d by uptake of ^3H -thymidine. BG, Background; □, spleen cells; ×, spleen cells treated with carbonyl iron; Δ, thymus cells.

Table 1 Response of spleen cells to various antigens

	PFC per culture $\pm$ s.e.		
	BG	MP	LPS
NNP-SRBC	153 $\pm$ 12	2,376 $\pm$ 125	2,297 $\pm$ 294
TNP-SRBC	177 $\pm$ 7	1,773 $\pm$ 104	2,290 $\pm$ 215
FITC-SRBC	90 $\pm$ 6	1,233 $\pm$ 151	1,480 $\pm$ 114
Dextran-SRBC	10 $\pm$ 1	133 $\pm$ 17	120 $\pm$ 20
SRBC	20 $\pm$ 6	113 $\pm$ 9	137 $\pm$ 9
HRBC	100 $\pm$ 30	516 $\pm$ 68	380 $\pm$ 55
DNA synthesis (c.p.m. per culture)	5,236 $\pm$ 276	25,697 $\pm$ 881	33,940 $\pm$ 1,060

Spleen cells from Nu/Nu mice were cultured in the presence of MP antigen (diluted 1/100) or LPS (100 µg) at a cell concentration of 5×10^6 cells per culture in medium with 5% foetal calf serum. Polyclonal antibody synthesis was measured after 2 d by detecting numbers of PFC to SRBC, HRBC and SRBC coupled with NNP, TNP, FITC or dextran. Determination of DNA synthesis was done in microcultures (5×10^5 cells per culture) pulsed for 24 h with 1 µCi ^3H -thymidine. BG, Background.

BALB/c background (Bomholtgaard, Ry, Denmark) and normal outbred guinea pigs. Spleen or thymus cells were cultured as described previously⁷ in Eagle's medium in Eagle's solution, or in RPMI 1640 medium with added L-glutamine, penicillin and streptomycin. Unless otherwise stated, serum was omitted from the cultures. DNA synthesis was measured and antibody-secreting cells were counted as before⁷. Briefly, 5×10^5 cells in 0.2 ml were cultured per well in microplates, usually for 48 h with a 24-h pulse of 1 µCi ^3H -thymidine or an 18-h pulse of 0.05 µCi ^{14}C -thymidine, and collected in a multiple cell culture collector

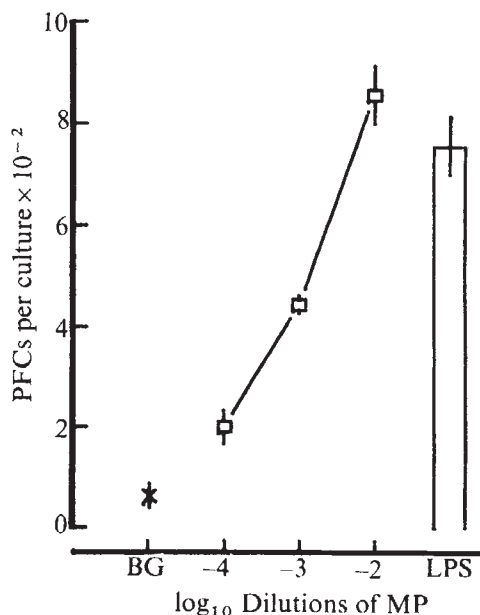


Fig. 2 Spleen cells from 6-week-old C57BL mice were cultured in the presence of MP or LPS (100 µg) at a cell concentration of 10^7 cells per culture, in serum-free medium. Polyclonal antibody synthesis was measured after 2 d by detecting numbers of PFC against SRBC coupled with NNP.

(Skatron A/S, Lierbyen, Norway). Radioactivity was measured in a liquid scintillation counter. To determine antibody secretion, 10^7 cells per ml per dish were cultured in plastic Petri dishes. Antibody-secreting cells were counted after 2 d of incubation in a modification of Jerne's haemolytic plaque assay, using as targets sheep red blood cells (SRBC), horse red blood cells (HRBC), or SRBC coupled with the following haptens or antigens: (4-hydroxy-3,5-dinitrophenyl) acetyl (NNP), trinitrophenyl (TNP), fluorescein isothiocyanate (FITC)⁷ or dextran⁸.

MP antigen was prepared as before² from organisms grown in broth on a glass surface. A concentrate of MP organisms was ultrasonicated and heated at 56 °C for 30 min to kill all viable organisms. The protein concentration of undiluted antigen was 2.5 mg ml⁻¹. LPS (obtained from Dr T. Holme, Stockholm) was extracted from *E. coli* 055 : B5 by the phenol water method. PPD (tuberculin RT 32) was obtained from Statens Seruminstitut, Copenhagen.

After addition of MP, spleen cells from normal mice responded with increased thymidine uptake (Fig. 1) as well as antibody secretion (Fig. 2). The peak of activation occurred on days 2 and 3 (data not shown). Removal of adherent cells only enhanced DNA synthesis (Fig. 1), and normal thymocytes did not respond. As the medium used for growing the MP organisms had no effect on either DNA synthesis or antibody secretion, we concluded that MP acts as a polyclonal activator for mouse spleen cells.

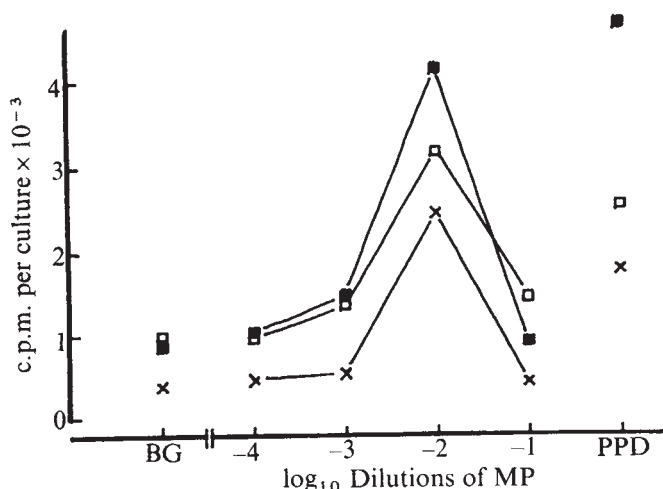


Fig. 3 Spleen cells from each of three guinea pigs were cultured in the presence of MP or PPD (100 µg) at a cell concentration of 5×10^5 cells per culture in serum-free medium. DNA synthesis was measured after 2 d by the uptake of ¹⁴C-thymidine. Each symbol represents the mean of triplicates of cells from one animal.

Adherent cells were not necessary for activation, and thymocytes did not respond, indicating that MP was a polyclonal B-cell activator. Indeed, spleen cells from Nu/Nu mice responded both by increased thymidine uptake and by producing increased numbers of antibody-secreting cells to various antigens (Table 1).

The multispecificity of the MP-induced antibody response and the fact that cells from pathogen-free mice responded makes it unlikely that stimulation was due to a previous *in vivo* infection by a microorganism cross reacting with MP antigens.

MP also stimulated DNA synthesis in spleen cells from normal guinea pigs (Fig. 3). Some animals showed a stronger response to MP than to PPD, whereas others responded better to PPD. Experimental MP infection in guinea pigs serves as a model for mycoplasma infection in man⁹, and so the guinea pig is a suitable animal for studies of the mitogenicity of MP *in vivo*.

An animal mycoplasma, *M. pulmonis*, has been shown to stimulate rat lymphocytes nonspecifically¹⁰. It is not known whether the responding cells were B or T lymphocytes. The stimulating factor of this mycoplasma was heat labile, in contrast to the mitogenic component of MP.

The results reported here do not provide information about the chemical nature of the MP component responsible for the mitogenic effect. The antigens eliciting specific antibodies to MP are located mainly in the mycoplasma cell membrane, which consists essentially of a lipid bilayer with proteins¹¹. The main serologically active MP components

are glycolipids with haptenic properties^{12,13}. Previous studies have shown that a lipid extract of MP, reacting with MP antisera in the complement-fixation test, did not stimulate sensitised or non-sensitised human peripheral blood lymphocytes². Further studies are necessary to elucidate the relationship between antigenicity and mitogenicity of various MP components.

Antibodies synthesised by mitogenically activated cells *in vitro* are most often of the IgM class⁶. Since MP infection in man is often followed by an increase in serum IgM, which is only partly due to specific anti-MP antibodies¹⁴, we suggest that this increase could in part be the result of MP-induced mitogenic activation of B cells. The fact that IgM antibodies directed to autoantigens are found after MP infection^{1,15} supports our suggestion. Mitogenicity of MP towards human lymphocytes has still to be firmly established, however.

In summary, our data show that MP, an important pathogen in man, is a polyclonal activator for mouse B lymphocytes and has a mitogenic effect on guinea pig spleen cells *in vitro*. The ability of MP to activate B cells polyclonally might influence the antibody response of patients and experimental animals infected with this microorganism. Finally, the fact that almost all substances which act as polyclonal B-cell activators are derived from microorganisms might indicate the importance of mitogenic activation for an effective immune defence¹⁶.

This work was supported by the Swedish Medical Research Council and the Swedish Cancer Society.

GUNNEL BIBERFELD

Central Microbiological Laboratory of
Stockholm County Council,
Department of Immunology,
National Bacteriological Laboratory,
Stockholm

EVA GRONOWICZ

Division of Immunobiology,
Karolinska Institute,
Stockholm, Sweden

Received February 18; accepted March 18, 1976.

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Purported difference between human T- and B-cell surface morphology is an artefact

BECAUSE of the previous inability to detect differences in lymphocyte morphology by light or transmission electron microscopy¹, reports that B and T lymphocytes examined with scanning electron microscopy (SEM) could be identified by their distinctive surface morphology have been met with interest²⁻⁶. In these studies, most 'villous' cells were identified as B lymphocytes and T lymphocytes were described as 'relatively smooth'. Some investigators have reported that T lymphocytes are more villous than B lymphocytes⁷⁻⁹; others, however, have not detected a difference in surface form among human^{10,11}, or murine lymphocytes¹²⁻¹⁴. The present study demonstrates that the

Table 1 Induction of smooth normal and leukaemic lymphocytes by an SEM preparative technique

	% Smooth cells	
	Fixed in suspension ¹⁵	Fixed after aspiration filtration ²⁻⁵
Normal peripheral blood*	0.7±0.3	94.5±0.7
Sezary's syndrome (T-cell leukaemia)†	1.6±0.3	81.2±4.5
CLL (B-cell leukaemia)†	2.0±0.3	85.8±5.4

*Mean ± s.e.; 4 experiments; 500 cells evaluated in triplicate samples per experiment (total $n = 6,000$).

†Mean ± s.e.; 500 cells evaluated in triplicate samples per patient ($n = 1,500$ per patient).

collection and fixation technique used in those studies that initially described the dichotomy in lymphocyte SEM morphology²⁻⁵, artefactually produces smooth cells.

The surface morphology of normal mononuclear leukocytes prepared for SEM by the aspiration filtration technique²⁻⁵ was contrasted with that of cells from parallel samples fixed in suspension (Table 1). When cells were fixed in suspension and processed on poly-L-lysine-coated glass coverslips, more than 97% of the cells were retained

for SEM observation¹⁵. Approximately 93% were covered uniformly with variable numbers of short microvilli, 6% were ruffled, and less than 1% was smooth (Table 1, Fig. 1a). When parallel cell suspensions were aspirated live on to the filters with subsequent fixation, however, approximately 95% of the leukocytes appeared relatively smooth (Table 1).

Leukaemic cells of B- or T-lymphocytic origin were analysed in the same manner. Ninety-nine per cent of the blood lymphocytes from a patient with Sezary's syndrome were considered to be T lymphocytes on the basis of the formation of spontaneous E rosettes with sheep erythrocytes and the failure to stain with fluoresceinated anti-human immunoglobulin. Approximately 80% of the purified lymphocytes from a chronic lymphocytic leukaemic (CLL) patient were B lymphocytes, by the criteria of the presence of surface immunoglobulin and the binding of heat-aggregated immunoglobulin and antigen-antibody complexes, and 99.5% failed to form E rosettes. Although 98% of the lymphocytes from these patients with leukaemias of B- or T-cell origin were villous or ruffled when fixed in suspension, 81% of the T-leukaemic cells and 86% of the CLL cells appeared smooth when aspirated live on to filters (Table 1 and Fig. 1b, c, e and f).

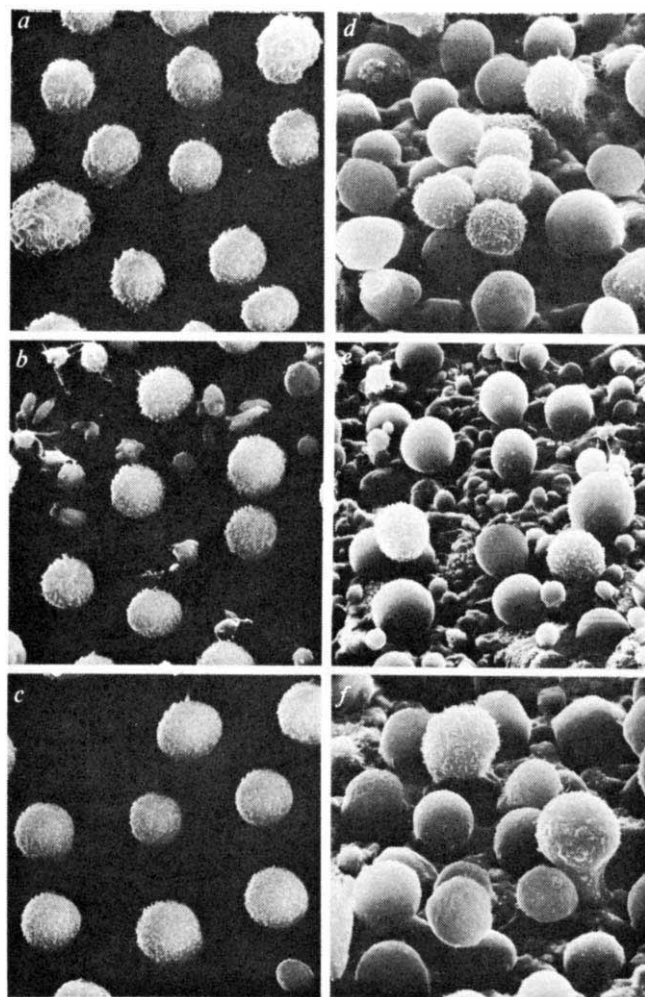


Fig. 1 Human peripheral mononuclear leukocytes were obtained from the defibrinated fresh blood of four normal donors by Ficoll-Hypaque density gradient centrifugation¹⁶. Differential counts indicated that on the basis of morphology 3% of the white cells were polymorphonuclear lymphocytes, 12% monocytes, and the remaining 85% lymphocytes. Leukaemic lymphocytes were obtained from the heparinised blood of a patient with CLL and by leukaphoresis from a patient with Sezary's syndrome, and processed as outlined for normal human peripheral blood. 95-98% of the cells in these populations were lymphocytes. Surface marker studies were carried out on these purified

leukaemic lymphocyte populations. E rosettes were carried out by a method published previously¹⁷. Surface immunoglobulin and aggregate binding and antigen-antibody complex binding were detected as described previously^{18,19}. Cell viability as determined by Trypan blue dye exclusion was greater than 95% for all preparations. Cells from each donor were processed for SEM by each of two methods. First, cells were fixed in suspension for 24-48 h at 22 °C by centrifuging 2×10^6 - 8×10^6 cells at 200g for 10 min, resuspending the cell pellet in 1.0 ml RPMI1640, and adding 0.8 ml 2.5% glutaraldehyde (Tousimis, Rockville, Maryland) in Sorensen's buffer (pH 7.2, 380 mosmol). This final 1% fixative concentration was selected to correspond to that previously utilised in studies reporting a difference in lymphocyte topography²⁻⁵. The fixed cells were then washed twice in Sorensen's buffer and processed on poly-L-lysine-coated glass coverslips as described previously¹⁵. The second method consisted of processing parallel samples by the aspiration filtration method according to Pollack *et al.*²⁻⁵. From 2×10^6 to 8×10^6 cells suspended in 5.0 ml of media were aspirated live on to silver membrane filters (13 mm diameter; Flotronics, Selas, Spring House, Pennsylvania) in Swinex holders (Millipore) with a peristaltic pump at a flow rate of 1.0 ml min⁻¹. When 0.5 ml of the suspension remained above the filter, 2.0 ml 1% glutaraldehyde in Sorensen's buffer were added dropwise. After 10 min, the filters were transferred to small plastic Petri dishes for continuing fixation in fresh fixative for 24-72 h. Fixation was carried out at 22 °C. All specimens were prepared in triplicate. Subsequently, the fixed cells on coverslips and silver filters were dehydrated through a graded series of ethanol and prepared by the critical-point drying method²⁰. Samples were processed entirely in the liquid phase to avoid air drying. The specimens were vacuum coated with gold-palladium on a tilting, rotating stage, examined with an Etce Autoscan microscope at 20 kV and 45° tilt, and photographed using Polaroid 55 film. The surface morphology of cells prepared by each of the two methods was classified into 'relatively smooth', 'villous', and 'ruffled' categories to correspond with previous descriptions of leukocyte surface morphology²⁻⁵. Percentages of smooth cells are reported in Table 1. *a-c*, Human leukocytes fixed in suspension, attached to poly-L-lysine-coated glass coverslips, and processed for SEM¹⁵. Cells are spherical and uniformly covered with short microvilli. Several ruffled cells and platelets are present. *d-f*, Parallel samples of populations illustrated in *a-c* prepared for SEM by the aspiration-filtration technique used in the descriptive studies²⁻⁵. The majority of cells shown have lost their villous surface topography. The field in *d* was selected from a filter with a high cell density to illustrate both villous and smooth forms. Ninety-five per cent of the cells were smooth on filters to which fewer cells (2×10^6) had been applied (Table 1). When cell density exceeds a monocellular distribution, cells not in direct contact with the filter tend to retain their villous structure (*d-f*). Smooth lymphocytes tend to be smaller and located within the crevices of the filter (*d-f*). Note the partially villous cell which seems to have been partly aspirated into a filter crevice (*f*). *a* and *d*, Normal peripheral blood leukocytes; *b* and *e*, cells from patient with CLL, B-cell leukaemia; *c* and *f*, cells from patient with Sezary's syndrome, T-cell leukaemia. $\times 1,750$.

By varying the number of cells applied to the filters, we have been able to vary further the ratio of smooth to villous cells in any given population. On filters where the cell density exceeded a monolayer, the surfaces of cells in direct contact with the substrate tended to be smoother than those of cells retained on the filters by way of attachment to other cells (Fig. 1d). Many of the smooth cells seemed to have been aspirated into crevices of the filter and such cells tended to be smaller than the more villous cells which were situated on top of the substrate or of other cells (Fig. 1d-f).

The present investigation demonstrates that smooth lymphocytes can be artefactually and variably generated from villous cells by the SEM preparative technique used in those studies that initially described B cells as villous and T cells as relatively smooth²⁻⁵. The alteration induced by this technique also seems to be nonspecific since it affects normal mononuclear leukocytes as well as leukaemic lymphocytes of both B and T origin. Thus, using the aspiration filtration method²⁻⁵, we have been unable to reproduce the observation that populations of T cells are predominantly smooth, whereas B cells are villous. A likely explanation for these differences lies in the way the experiments of Polliack *et al.*²⁻⁵ were carried out and the manner in which the results were interpreted.

For example, we have demonstrated that the technique of aspiration filtration²⁻⁵ smooths the surfaces of living cells in direct contact with the filters and aspirates portions of some of the cells into the filter crevices. Thus, it seems that the surface characteristics of the final population are likely to depend on the specific conditions used, such as: duration of contact with substrate, the negative pressure applied to the filter, and/or cell density. It is not clear that these important variables were controlled adequately in the previous experiments²⁻⁵. In addition, control of other important factors, such as the conditions in which cells were handled before aspiration filtration has not been reported by proponents of the T-B surface structure dichotomy²⁻⁵. Cold temperature, for example, has been shown to smooth the surface architecture of lymphocytes^{21,22}. Normal T lymphocytes induced to form E rosettes at 4 °C are relatively smooth^{3,23}, but are villous when E rosettes are formed at 20 °C (refs 8 and 24). R.B. (unpublished) has demonstrated that lymphocytes from the same E-rosetting T-cell population are villous at room temperature and relatively smooth at 4 °C. When all these variables are controlled we observed no difference between the surface morphology of B and T lymphocytes. Thus it is possible that the initial results, that ratios of T to B cells in normal human peripheral blood seemed to correlate with surface morphology, were secondary to one or any combination of these variables and erroneously attributed to a structure-function relationship. Differences in the handling of various samples without regard to these variables could have led to a perpetration of the error with rosetted cells, cell lines and leukaemic populations.

The investigators correlating ratios of villous to smooth cells with proportions of B and T lymphocytes^{2-5,25} determined by surface marker studies also failed to appreciate that there is no *a priori* reason to assume that the sets of surface morphological characteristics (that is, smooth and villous) are coextensive with the sets of functional classes of lymphocytes identified by surface markers (that is, T and B cells). This is particularly true when the populations used for markers may not have been comparable with those used for SEM (see ref. 26 for discussion). The analysis of individual cells fixed in suspension and positively identified by SEM surface markers demonstrated no difference in the surface morphology of surface immunoglobulin-positive and -negative cells^{12,13}.

A study using Hoffmann modulation contrast light microscopy (40× Nikon achromat objective; NA 0.65)

with a maximal theoretical resolution just at or below the limits needed to delineate lymphocyte microvilli, failed to detect surface microvilli on most murine lymphoid cells²⁴. When living human lymphocytes from monocyte-depleted Ficoll-Hypaque preparations, however, were processed at room temperature and examined with light microscopy (100 × Zeiss Planachromat objective; NA 1.25), 98% were villous (unpublished). These results are consistent with the report²⁷ that murine θ -positive and surface immunoglobulin-bearing lymphocytes stained with fluoresceinated ligands are equally villous.

In conclusion, it is not possible to identify reliably classes of lymphocytes *in vitro* exclusively on the basis of their SEM surface topography. Our observations challenge the concept of 'villous B' and 'smooth T' lymphocytes and indicate the necessity for a more critical approach to the preparation of cells of all types for SEM. These results do not exclude the possibility that subpopulations of lymphocytes may demonstrate distinctive differences in surface morphology with higher resolution electron microscopy. Nor do they preclude the possibility that lymphocyte subpopulations might respond differentially to certain micro-environmental manipulations with distinctive changes in surface morphology²⁸. Because it has been amply demonstrated that lymphocyte surface morphology is dynamic and exquisitely susceptible to changes in *in vitro* conditions^{7,10,24,26-28} it is likely that lymphocytes undergo continual alterations in their shape and form as they mature, divide, circulate, migrate and interact with surfaces and other cells as they function *in vivo*.

We thank Drs S. Ben-Sasson, C. Berard, P. Henkart, W. Terry, T. Triche and J. Wunderlich for support and suggestions; and Judy Steckel and Ralph Isenburg for assistance.

ELAINE ALEXANDER
SHEILA SANDERS
RAUL BRAYLAN

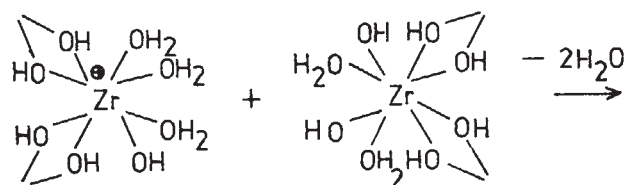
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received December 18, 1975; accepted March 23, 1976.

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Microbial cells living immobilised on metal hydroxides

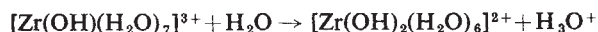
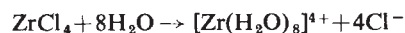
THE immobilisation of enzymes by attachment to water-insoluble materials has received considerable attention recently¹, both for academic reasons and for possible industrial applications. A logical extension of this approach, especially where multi-stage enzymic reactions are being considered, is the immobilisation of microorganisms, which are often the source of many enzyme preparations. The advantages of such an approach are immediately obvious. The tedious and time consuming procedures for enzyme extraction and purification are instantly eliminated, cofactors and coenzymes are readily at hand, the cellular enzymes are often organised into the requisite metabolic pathways and problems associated with enzyme instability may also be avoided. Furthermore, the use of immobilised cells would avoid the problem in industrial processes of separating the product from the enzyme. The immobilisation of cells by the standard glutaraldehyde procedure, however, causes their decease, and entrapment in polyacrylamide gel is commonly used instead to achieve immobilisation^{2,3}. This method produces a minimum of interaction between the microbial cell and the insoluble matrix, thus maximising the probability of the cell's survival. On the other hand, the availability of the cell to any intended substrate is seriously reduced, since it can reach the cell only by diffusion. An alternative approach of immobilising cells on collagen, by the formation of a stable network of multiple ionic (salt) linkages, hydrogen bonds and Van der Waals' interactions has been successful⁴. We have now pursued an independent approach and report here the successful immobilisation of cells on the hydroxides (hydroxyoxides) of titanium (IV) and zirconium (IV) by a chelation process.



Titanium hydroxides are insoluble over the normal physiological pH range, and acting as enzyme immobilisation matrices give good retention of enzymic activity^{5,6}, they thus seem to have little or no effect on the function of biologically active molecules. If enzymic activity, which is extremely sensitive to conformational changes in the enzyme molecule, is not seriously affected by immobilisation, then there seems to be little reason why cell walls should be disrupted or destroyed by this process and the cells themselves, therefore, have a good chance of remaining viable. Zirconium has an outer electron configuration of $4d^25s^2$ and occurs almost exclusively in its compounds in the $4+$ oxidation state. Bonding to the maximum possible extent results in a maximum coordination number of 8. The

zirconium ion can use various hybrid orbitals to give strongly directional bonds. For eightfold coordination, the orbitals used are d^4sp^3 , leading to a dodecahedral arrangement, or d^5p^3 , giving a square antiprismatic configuration⁷. In the latter, the lone pairs of electrons in the ligands form coordinate bonds with the vacant 4d and 5p orbitals of the zirconium ion, which are essentially covalent, involving the sharing of the lone pair of the oxygen atom.

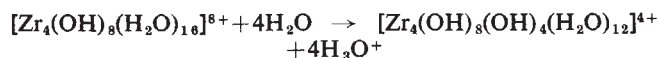
The behaviour of zirconium complexes in aqueous solution is characterised by hydrolysis and polymerisation. When zirconium (IV) chloride is dissolved in water the reactions which occur can be formulated as below



The final complex cation is then able to polymerise to give a tetrameric complex



This tetrameric complex has been shown⁸ to be present in the aqueous solution, which has acidic properties, the H^+ activity of a mM solution being almost equal to that of mM HCl (ref. 9). This arises from the hydrolysis of the tetrameric complex



The charge on the complex is thus reduced to $4+$ and, depending on the acid concentration, can be reduced further. Each

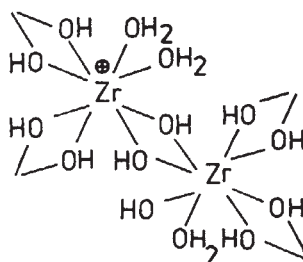
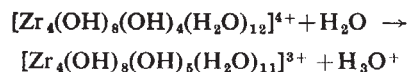


Fig. 1 The polymerisation of hydrolysed zirconium complexes.

of the four zirconium ions can hydrolyse separately



This results in one neutral group in the tetrameric complex. Assuming that the neutral group is localised on one of the zirconium atoms, the other three retaining their single positive charges, then this can be the starting point for a polymerisation reaction. The neutral site reacts with a singly charged site of another tetrameric complex so that the zirconium ions are connected by two hydroxide bridges (Fig. 1). The degree of polymerisation increases as the acid strength decreases⁹ and

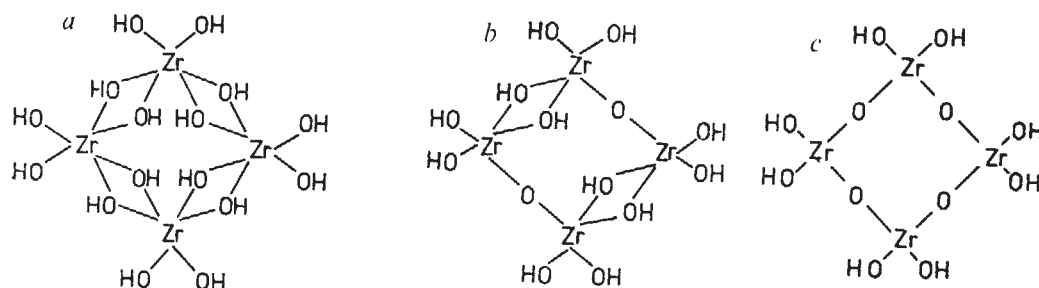
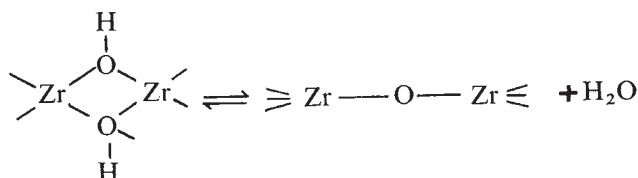


Fig. 2 a, b, c, Structures of zirconium hydroxide.

so addition of alkali to neutralise the acidic solution formed on dissolution of zirconyl chloride also increases the degree of polymerisation. When neutralisation is continued to pH 3, a precipitate begins to form. At the equivalence point (pH 9) precipitation is complete, the gelatinous precipitate being zirconium hydroxide (also known as hydrated zirconium dioxide or hydrous zirconia).

We propose that the precipitate contains a variety of compounds. The formation of the hydrated oxide is preceded by a stage involving the production of a true hydroxide containing hydroxyl groups¹⁰. The conversion of the hydroxide into 'hydrous zirconia' takes place very rapidly and irreversibly so that in practice the gelatinous precipitate is not the hydroxide.

Zaitsev¹¹, however, has shown that freshly precipitated zirconium hydroxide contains four hydroxo-groups for every zirconium atom and therefore that the compound with the formula $\text{Zr}(\text{OH})_4 \cdot n\text{H}_2\text{O}$ can exist. Its stability is low, and on ageing it is converted into the so-called zirconyl hydroxide, containing two hydroxo-groups for every zirconium atom: $\text{ZrO}(\text{OH})_2 \cdot n\text{H}_2\text{O}$ (ref. 11). When this is dried in the cold to a powder, it is converted into hydrated zirconium dioxide, having the formula $\text{ZrO}_2 \cdot x\text{H}_2\text{O}$ and containing no hydroxyl groups. Only two hydroxyl groups per zirconium atom of freshly precipitated zirconium hydroxide react with acid, the other two remaining inactive because double hydroxo- (or olate) bridges are formed between the zirconium atoms. The structure of the freshly precipitated hydroxide is thus extremely likely to be that depicted in Fig. 2a, together with an indefinite number of water molecules. This structural formula represents the main unit of the hydroxide, but in certain circumstances some of these tetrameric molecules may be joined together by oxo- or olate bridges. Conversion of a double olate bridge into an oxo-bridge is possible¹² by the following mechanism



suggesting that two other zirconium hydroxides may exist having zirconium atoms joined alternatively by double olate and oxo-bridges or zirconium atoms joined only by oxo-bridges. The possible structures of these hydroxides are shown in Fig. 2b and c; Fig. 2b represents a hydroxide having the formula $(\text{OH})_3\text{Zr}-\text{O}-\text{Zr}(\text{OH})_3 \cdot n\text{H}_2\text{O}$ and Fig. 2c corresponds to the hydroxide with the formula $\text{ZrO}(\text{OH})_2 \cdot n\text{H}_2\text{O}$. The infrared absorption spectra of these two compounds indicate that they do not contain the $\text{Zr}=\text{O}$ group, and so must be cyclic polymers of the type indicated. We therefore conclude that in precipitated hydrous zirconium hydroxide there is an abundance of hydroxy groups replaceable by ligands.

Since the immobilisation process for a number of metal hydroxides is envisaged as involving the replacement of hydroxyl groups¹³ on the surface of the metal hydroxide by suitable ligands from enzyme or cell, resulting in the formation of partial covalent bonds, we conclude that zirconium hydroxide should be suitable for immobilisation phenomena. In the case of enzymes, such ligands could be the side-chain hydroxyls of L-serine or L-threonine, the carboxyls of L-glutamic acid or L-aspartic acid and the ϵ -amino group of L-lysine residues, (Fig. 3), oxygen-containing ligands being preferred to those containing nitrogen¹⁴. In the case of cells, the structural complexity of the cell wall ensures the availability of a great diversity of suitable ligands from both protein and carbohydrate moieties.

Samples (each of 1.3 mmol) of the metal hydroxides for use in cell immobilisation were prepared from solutions of their tetrachlorides (titanium tetrachloride 15% w/v in 15% w/v hydrochloric acid (BDH) and zirconium tetrachloride (BDH) 0.65 M in 1.0 M hydrochloric acid) by the slow addition of

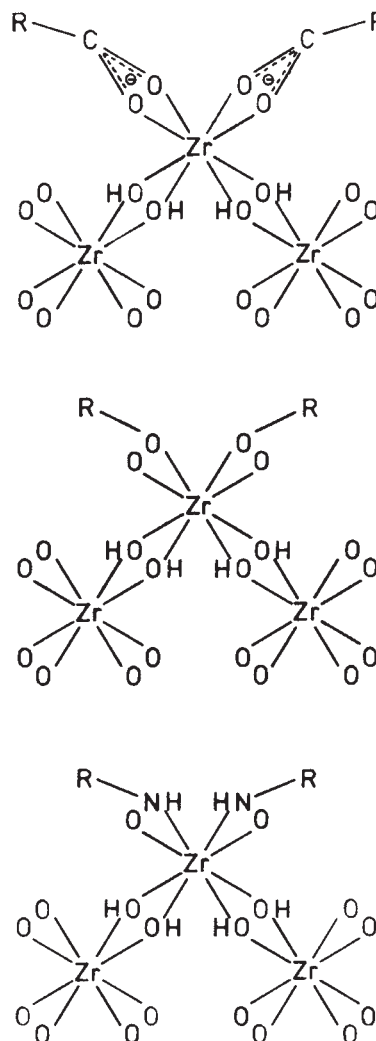


Fig. 3 Projected structures of the complexes of zirconium hydroxide with carboxyl, hydroxyl and amino groups respectively.

2.0 M ammonium hydroxide to neutrality (pH 7.0). The samples were washed with saline solution (0.9% w/v, 3×5.0 ml) to remove ammonium ions and then used for cell immobilisation studies as below.

The procedure for cell immobilisation is very simple and is illustrated by the following example. A suspension (in 10 ml 0.9% w/v saline) of *Escherichia coli* cells (A_{600} 0.216) was mixed with a sample of the metal hydroxide prepared as above and agitated gently for 5 min at room temperature. The mixture was then allowed to stand at room temperature and the suspension settled out, leaving a clear supernatant (A_{600} 0.022) which was practically devoid of microorganisms (shown by microscopy). The immobilised cell preparation was consolidated by centrifugation at low speed and removed from the supernatant for further examination.

Saccharomyces cerevisiae and *E. coli* cells were immobilised in this manner and the preparations were examined for continued viability by measurement of their oxygen uptake (at 25 °C in aerated 0.2 M sodium acetate buffer pH 5.0, or 0.9% w/v saline by use of an oxygen electrode (Pye Unicam Ltd). The rate of oxygen uptake of the immobilised cells was ~30% of that of the same number of free cells. This result showed that respiration of the cells could continue when the cells were immobilised. The reduced rate of oxygen uptake is probably caused by the restriction by the metal hydroxide of access of aerated buffer to the cells and a decrease of the area of cell surface available for oxygen transfer.

To show that the cells were firmly attached to the surface of the metal hydroxide and not just loosely trapped in the gelatinous

matrix, a number of arguments are invoked. Free cells of *E. coli* are comparatively small and cannot be centrifuged down to any significant extent in the centrifugation conditions used for collecting the immobilised cells; they are not particularly robust and so any disruption process which had occurred would, therefore, have rendered them inactive and unable to respire. Solutions of bicarbonate, phosphate, fluoride (and so on) ions which have been shown⁶ to remove loosely bound proteinaceous and other materials from zirconium (IV) hydroxide were singularly ineffective in the attempted release of the immobilised cells from the matrix.

To show further that the cells were firmly attached to the metal hydroxide, a different microorganism, *Serratia marcescens* was employed. When incubated in nutrient medium at 25 °C, this organism produces a distinctive red colouration, which enables the immobilised cells to be distinguished readily from those of any other contaminating microorganisms and also obviates the need for sterile experimental conditions.

On adding cultures of *S. marcescens* to zirconium (IV) and titanium (IV) hydroxides, the red colouration (that is, the cells) became associated with the insoluble matrix, the supernatant and subsequent washings (with saline solution) being almost cell free, thus demonstrating the strength of the cell-metal hydroxide interaction. When samples of these immobilised cells were added as a small inoculum to fresh culture medium, growth was observed (as detected by the large increase in numbers of red cells). Since at no time throughout extended studies of the growth of *S. marcescens*, in various conditions of humidity, oxygen tension, temperature and medium composition and ultraviolet irradiation-induced mutation, was release of colour from the cells observed, this was taken as evidence that the cells had suffered no deleterious effects on immobilisation.

It was also found that cells could be immobilised at lower pH, titanium (IV) hydroxide being more effective for this purpose, in the range studied (pH 2–5). This phenomenon is useful since not all microorganisms exist in a neutral pH environment (for example, *Lactobacillus* and *Acetobacter*) and enabled us to produce a small scale immobilised cell reactor. *Acetobacter* cells, immobilised on titanium (IV) hydroxide, were able to produce acetic acid from an alcoholic medium in a continuous reactor, often at rates higher than those obtained when free cells were used (a basic rate 87 g acetic acid per day (86% conversion) increased to 263 g per day (99% conversion) after immobilisation), thus demonstrating the practicability of this immobilisation method. If this simple means of cell immobilisation was applied to other microorganisms it could well result in further immobilised cell reactors of this sort, for the selective production of commercially important biochemical and pharmaceutical compounds. Furthermore, we conclude from the results that immobilised cells can retain their activity for a matter of weeks at least.

As with immobilised enzymes, immobilised cell systems have been applied to very few industrial processes, partially because of the inertia of established methods and partially because of the increased difficulty of operating such systems. It seems certain, however, that the inherent advantages of these systems will eventually prevail and there will be an increasing use of immobilised systems by the pharmaceutical, chemical and food industries.

The material of this publication is included in US Patent 3,912,593, British Patent Application No. 41436/72.

JOHN F. KENNEDY
S. ALAN BARKER
JOHN D. HUMPHREYS

Department of Chemistry,
University of Birmingham,
P.O. Box 363, Birmingham, B15 2TT, UK

Received December 22, 1975; accepted March 4, 1976.

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Phenotypic difference between *uvrA* and *uvrB* mutants of *E. coli*

THE synthesis of colicin E1, an antibiotic protein, by cells of *Escherichia coli* is coded for by a bacterial plasmid, colicinogenic factor E1 (ColE1). The synthesis is induced by agents such as ultraviolet light¹ or mitomycin C (mit C)². Ultraviolet and mit C are known to modify the base portions of DNA to form pyrimidine dimers³ and cross linkages between complementary strands⁴, respectively. Damage to DNA caused by ultraviolet or mit C is repairable by a similar molecular mechanism^{5,6}, but the relationship between these repair systems and the induction process of ColE1 synthesis has not been established unequivocally. We have analysed the induction of the colicin by ultraviolet and mit C in various ultraviolet-sensitive mutants to which the colicinogenic factor was transferred. Among the mutants tested, the *recA* mutant did not synthesise the colicin in response to ultraviolet or mit C as reported previously⁷. In addition, however, we have now found that the induction of the colicin synthesis is greatly delayed in the *uvrB* mutant, but is normal in the *uvrA* mutant.

Both *uvrA* and *uvrB* mutants are deficient in removing pyrimidine dimers, or cross linkages caused by mit C,

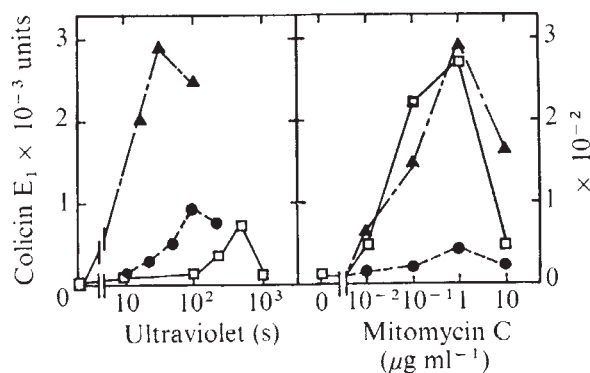


Fig. 1 Effect of varying doses of ultraviolet light and mitomycin C on the induction of colicin E1 synthesis. The following strains of *E. coli* K12 were used: AB1157 (*uvrA*⁺, *thr*⁺, *pro*⁺, *leu*⁺, *his*⁺, *arg*⁺, *thi*⁺, *lac*⁺, *gal*⁺, *ara*⁺, *xyl*⁺, *mtl*⁺, *tsx*⁺, *str*^r, *F*⁻), AB2437 (*uvrA*⁻, *his*⁺, *thi*⁺, *str*^r, Hfr), and AB1885 (AB1157 *uvrB*⁻). ColE1 DNA was transferred to these strains according to the method previously described¹⁴. For the growth of bacteria, minimal salt media (M-9) supplemented with vitamin-free casamino acids (0.15%), thiamine (10 μg ml⁻¹) and glucose (0.4%) were used¹⁴. Cells of each strain which were aerobically grown to log phase (1 × 10⁹ cells ml⁻¹) were washed and incubated without aeration in the above synthetic media at 37 °C in the dark after addition of mit C or irradiation with ultraviolet light. After 2 h the incubation mixtures were treated by sonic oscillation and the colicin activities were determined as previously described¹⁴. The colicin activity of untreated cells (less than 20 units per A₆₆₀) was subtracted from activities obtained. For ultraviolet induction, the bacterial suspension (2.5 ml) was irradiated in 9-cm diameter Petri dishes with a 6 W low pressure mercury National germicidal lamp from the height of 1 m. In these conditions, irradiation for 60 s is equivalent to 30 erg mm⁻². □, *uvrA*⁻; ▲, *uvrB*⁻; ●, *uvrB*⁻.

Table 1 Time course of the induction of colicin E1 synthesis by mitomycin C and decarbamoyl mitomycin C

Strain	mit C-induced colicin E1 activity at					DCmit C-induced colicin E1 activity at	
	0	1	2	4	8	0	2
			(h)				(h)
AB1157 (ColE1) <i>uvr</i> ⁺	0	25	100	69	54	0	90
AB2437 (ColE1) <i>uvrA</i> ⁻	0	50	100	42	26	0	100
AB1885 (ColE1) <i>uvrB</i> ⁻	0	5	10	33	80	0	81
KMBL49 (ColE1) <i>uvr</i> ⁺	0	56	100	100	100	0	88
KMBL90 (ColE1) <i>uvrB</i> ⁻	0	1	20	38	53	0	70
KMBL101 (ColE1) <i>uvrB</i> ⁻	0	14	32	32	70	0	68

AB1157 (ColE1), AB2437 (ColE1), and AB1885 (ColE1) were described. KMBL90 *dar*₁⁻ (*uvrB*⁻) and KMBL101 *dar*₆⁻ (*uvrB*⁻) were derived from KMBL49 (KA22 *dar*⁺, *thr*⁻, *leu*⁻, *thi*⁻, *pyr*⁻, *thy*⁻)¹¹. For the growth of these KMBL strains, M-9-glucose-casamino acids media (see Fig. 1) were further supplemented with thymine (20 µg ml⁻¹), uridine (200 µg ml⁻¹), and biotin (4 µg ml⁻¹). Transfer of ColE1 DNA to these strains was made from *nadC*13 (ColE1) with the aid of W3110 prototroph F⁺. The concentration of mitomycin C or decarbamoyl mitomycin C was 1 µg ml⁻¹. Conditions for induction and determination of colicin E1 activity have been described previously.

compared with the *uvr*⁺ strain^{5,6,8} and are considered to be defective in the endonucleolytic steps of excision repair⁹. Apart from the difference at the genetic locus^{10,11}, no phenotypic differences between these two mutants have been found with respect to the following points at least: sensitivity to ultraviolet, mit C (ref. 5) and X rays¹⁰; the capacity of ultraviolet or mit C to induce mutations^{6,12}; the ability to reactivate ultraviolet-irradiated T₁ or λ phage^{5,13}; the inducibility of prophage by ultraviolet or mit C (ref. 13); and the absence of endonuclease activity⁹. As far as colicin E1 induction was concerned, however, the *uvrB* mutant behaved differently from the *uvrA* mutant and this provides evidence for the first time that the functions of *uvrA* and *uvrB* genes are not completely identical.

Cells of the *uvrA* and *uvrB* mutants and a *uvr*⁺ strain¹⁰ which were made colicinogenic were treated with various doses of ultraviolet and mit C and incubated for 2 h in the standard induction conditions¹⁴. In the case of ultraviolet induction, both mutants synthesised greater amounts of colicin E1 in response to lower doses of ultraviolet than did the wild type (Fig. 1). In contrast, on mit C induction, little colicin E1 was produced in *uvrB* mutants at any concentration of mit C, whereas colicin E1 synthesis in *uvrA* mutants was almost the same as in the *uvr*⁺ strain (Fig. 1). The optimal concentrations of mit C for induction were the same for these mutant strains, whereas the optimal doses of ultraviolet for induction were different in the two strains. Ranking the mutants according to the ultraviolet dose required for maximum colicin induction correlated with the order of sensitivity to ultraviolet-induced killing; the *uvrA* mutant was the most sensitive to ultraviolet and the optimal dose for colicin induction was also the lowest.

In the *uvrB* mutant, however, when the cells were incubated for a longer period, the colicin was gradually synthesised (Table 1). After 8 h, the mutant produced almost the same amount of colicin E1 as the *uvr*⁺ strain. The same findings were obtained from other *uvrB* mutants such as KMBL90 (ColE1) and KMBL101 (ColE1)¹¹. Thus, it can be concluded that colicin E1 synthesis was greatly delayed in *uvrB* mutant.

Viable cell number of the *uvrB* mutant decreased to 1% after a 30-min incubation with mit C (1 µg ml⁻¹). In addition, when the *uvrB* cells were incubated for 30 min with mit C, followed by washing and additional incubation in the fresh medium without mit C, the same time course of colicin E1 synthesis was obtained as without treatment. These observations suggest that the first step of the action of mit C is already complete within 30 min of incubation in the *uvrB* mutant and that a certain process after this step to colicin E1 protein synthesis is impaired.

Decarbamoyl mitomycin C (DCmit C), a monofunctional alkylating agent, could induce colicin E1 synthesis at a normal rate in all of the above *uvrB* mutants (Table 1). DCmit C attaches to a single strand of DNA and does not cross link between the strands⁵. In addition, the DNA damage caused by the monofunctional mit C was repairable

by an excision repair system similar to that for ultraviolet-irradiated DNA. Thus, the delayed response of the colicin synthesis in *uvrB* mutant was associated with the formation of the cross linkages between the DNA strands.

Although the *uvrA* mutant lacks the ability to repair DNA damage caused by mit C, DCmit C and ultraviolet^{5,9} by excision, the mutant was induced to synthesise colicin E1 by these agents. It is, therefore, unlikely that the excision repair process itself is directly involved in colicin induction. In these mutant strains, another repair mechanism, the post-replicative repair system, will operate in some cases¹⁵. The operation of post-replicative repair seems to be essential for colicin induction, since neither mit C nor ultraviolet could induce colicin E1 synthesis in a *recA* mutant. The *recA* gene is believed to control the whole process of the post-replicative repair system¹⁵.

We thank Drs K. Mizobuchi and K. Suzuki for bacterial strains and Dr M. Tanaka for DCmit C. This research was supported in part by the Scientific Research Fund of the Ministry of Education of Japan.

NOBUO SUZUKI
ATSUSHI NAKAZAWA

Department of Biochemistry,
Chiba University School of Medicine,
Inohana, Chiba, Japan

Received January 12; accepted March 11, 1976.

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An intracistronic region of gene A of bacteriophage φX174 not involved in progeny RF DNA synthesis

GENE A of the single-stranded DNA bacteriophage φX174 has a key position in the replication of the first double-stranded replicative form (parental RF) to progeny RF DNA molecules. The action of gene A manifests itself in the introduction of a nick in the viral strand of the parental RF (ref. 1), which seems to be a condition for the RF DNA replication. The nick is located within gene A (ref. 2)

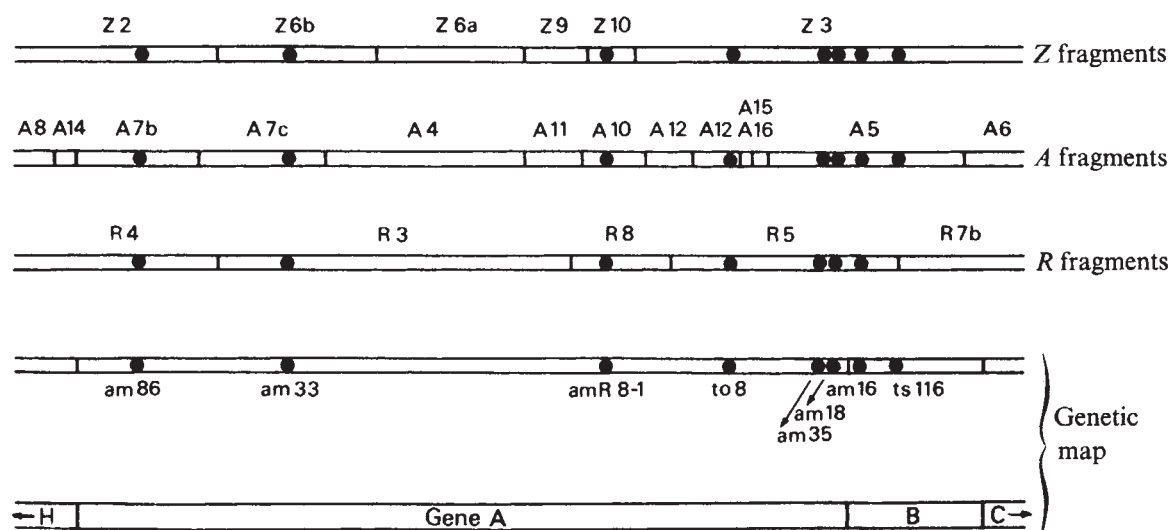


Fig. 1 Genetic map of the gene *A* and gene *B* region.

and is achieved by a *cis*-acting gene product^{3,4}. It has been shown that gene *A* codes for two overlapping proteins, *A* and *A'* (molecular weights 35,000 and 60–65,000, respectively)^{5,6}. The molecular weight of the large protein corresponds to the size of gene *A* as determined using restriction enzyme fragments⁷. The large *A* protein may be involved in achieving the nick⁸, the small *A* protein in the shut-off of the host DNA replication⁹. We present here genetic evidence that gene *A* consists of three different regions. Two of them are involved in double-stranded DNA replication. They are physically separated by a third region, characterised by mutations that leave double-stranded DNA synthesis unaffected.

We have isolated a group of conditional lethal nonsense mutants of ϕ X174 which could not be assigned unambiguously to one of the known genes. In complementation tests these mutants complemented well with mutants of genes *A*, *C* up to and including *H*, but the complementation behaviour with mutants of gene *B* was aberrant. This

is shown in Table 1 for *to8*, one of the mutants¹⁰ of that group. No complementation could be found between *to8* and the amber mutants of gene *B*, whereas complementation with the gene *B* *ts* mutants did occur, although marginally (the temperature seemed to be very critical to the selfing of these *ts* mutants).

In the first instance we supposed that *to8* belonged to a new cistron, located (in terms of translation) distally of and adjacent to gene *B* (ref. 12). If so, the complementation behaviour could be explained by a polarity effect of the amber mutants of gene *B* over the mutants of the new cistron. After the development of the new technique of localisation of mutants with the help of restriction enzyme fragments⁷, however, we found (Table 2) that the *to8* mutation is covered by the R5 fragment made by the restriction enzyme *Hind*II from *Haemophilus influenzae* Rd, the Z3 fragment made by *Hae*III from *H. aegyptius*, one of the four A12 fragments made by *Alu*I from *Arthrobacter luteus*, and the R5A12 fragment (= the A12

Table 1 Complementation of *to8* with mutants of all the known ϕ X174 genes

Complementation of <i>to8</i> with		Temperature (°C)	Burst sizes			Complementation behaviour
Test mutant	Gene		Mixed infection	Selfing <i>to8</i>	Selfing test mutant	
<i>am86</i>	<i>A</i>	30	42	0.32	0.21	+
<i>am33</i>	<i>A</i>	30	45	0.32	0.26	+
<i>am35</i>	<i>A</i>	30	34	0.32	0.24	+
<i>am18</i>	<i>A</i>	30	26	0.32	0.26	+
<i>am16</i>	<i>B</i>	30	0.28	0.32	0.22	—
<i>amH210</i>	<i>B</i>	30	0.22	0.32	0.06	—
<i>ts116</i>	<i>B</i>	39	0.84	0.05	0.31	—
		38	4.2	0.37	0.51	?
		37	9.4	0.31	2.4	?
<i>ts6</i>	<i>B</i>	39	2.1	0.06	0.05	+
		38	11	0.37	0.06	+
		37	18	0.31	2.8	?
<i>och6</i>	<i>C</i>	37	58	0.05	0.76	+
<i>am42</i>	<i>D</i>	39	23	0.01	0.01	+
<i>am3</i>	<i>E</i>	39	64	0.03	0.10	+
<i>ts41D</i>	<i>F</i>	41	1.9	0.02	0.02	+
<i>am9</i>	<i>G</i>	41	18	0.01	0.01	+
<i>ts4</i>	<i>H</i>	39	53	0.02	0.35	+

Escherichia coli C (*su*[−]) cells were grown with aeration to log phase, centrifuged and resuspended in fresh medium to a concentration of 10^8 – 5×10^8 cells ml^{−1}. Ten minutes after addition of KCN (final concentration 0.003 M) the phage mutants were added at a multiplicity of three of each in the mixed infection and of six in the selfings. After a 10-min adsorption period the unadsorbed phage were inactivated by incubation with ϕ X174 antiserum for 10 min. The adsorption mixture was diluted 10^4 times in prewarmed medium and after a 1-h growth period the yield of mutant particles was determined by plating on *E. coli* HF4712 (*su*⁺) at 30 °C. In the combination with *och6* the growth period was shortened to about 30 min, and the yield of the mixed infection and of the selfings was scored on *E. coli* WWU (*su*VIII) at 30 °C. Growth medium for *E. coli* WWU was TPG medium¹¹ supplemented with 0.05 g methionine, 0.05 g tryptophan, 0.04 g proline, 0.04 g thymidine and 0.03 g cytidine l^{−1}. Other growth media were as described previously¹⁰. Each combination of mutants was tested in at least two different experiments. Test mutants were provided by Dr R. L. Sinsheimer *et al.*, except for *amH210* which was provided by Dr M. Hayashi.

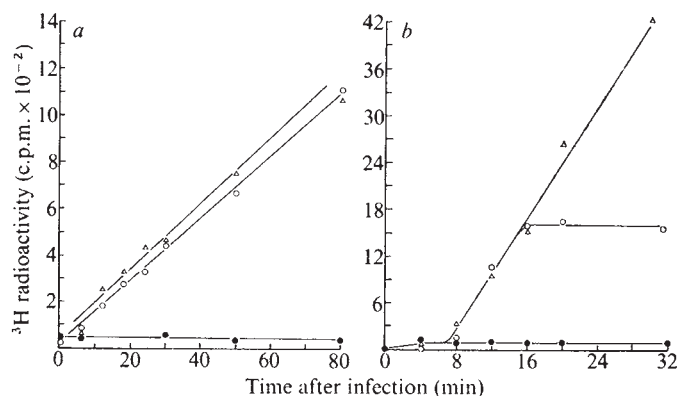


Fig. 2 Measurement of DNA synthesis. *E. coli* HF4704 (*thy*⁻, *uvrA*, *su*⁻) was grown in TPG medium¹¹ containing 200 µg casamino acids and 2 µg thymine ml⁻¹ to a concentration of 3 × 10⁸ cells ml⁻¹ and treated with mitomycin C (50 µg ml⁻¹) for 25 min at 37 °C in the dark to block host DNA synthesis. The cells were centrifuged down and resuspended in fresh TPG medium containing 200 µg casamino acids and 1 µg thymine ml⁻¹, plus 35 µg chloramphenicol (CAM) ml⁻¹ (a) or without CAM (b). After a 10-min incubation period at 37 °C ³H-thymidine (specific activity 40 µCi µg⁻¹) was added to a final concentration of 2.5 µCi ml⁻¹, simultaneously with the phage (multiplicity of infection = 3). DNA synthesis was measured by the incorporation of ³H-thymidine into TCA-precipitable material. The precipitation was accomplished by adding 100 µg bovine serum albumin as a carrier to a 0.5-ml sample. After 20 min in the cold the precipitate was spun down and washed twice with cold TCA, solubilised in 1 ml 0.55 N KOH and counted for radioactivity in a liquid scintillation counter. A correction was made for non-infected cells. ○, *to8*; ●, *am33*; △, *am3*.

The observation that *to8* is not affected in the progeny RF DNA synthesis, although it maps within gene *A*, leads to a division of gene *A* into three functionally distinct regions. Two regions are involved in the progeny RF DNA replication. They are separated by a third region in which mutations permit normal progeny RF DNA synthesis. Also, these mutants do not show the asymmetry in complementation as mutants in both the other regions do¹⁰.

How can we interpret these results in terms of the functioning of gene *A*, considering our present knowledge? Both *am86* (*A*) and *am18* (*A*) interfere with the synthesis of the 60,000 molecular weight protein⁹, although they are separated by the *to8* region that seems to have a different function. The complementation results indicate three separate genes, two of which are involved in the progeny RF DNA synthesis in such a way that mutants in both functions do not complement each other. The 60,000 molecular weight protein could then be a read-through product, without any function. This, however, is in conflict with the endonuclease activity ascribed to this protein⁹.

On the basis of our experiments we prefer the hypothesis of three separate cistrons. Probably the real situation is even more complex, considering the finding that *to8* can be complemented by the *ts* mutants of gene *B*, but not by its amber mutants. This suggests a functional relationship between the products of the genes *A* (*to8* cistron) and *B*. Work is in progress to elucidate these phenomena.

We tried to estimate the size of the *to8* region by inducing amber mutations in that part of gene *A* by the technique of fragment mutagenesis¹⁸. We found three mutants which behaved like *to8* in the complementation

Table 2 Localisation of *to8* mutation with restriction enzyme fragments

R fragment	Ratio wild type-total	Z fragment	Ratio wild type-total	A fragment	Ratio wild type-total	R5 digested with <i>AluI</i>	Ratio wild type-total
R3	7.8 × 10 ⁻⁵						
R8	1.7 × 10 ⁻⁵	Z10	4.5 × 10 ⁻⁵				
R5	1.2 × 10 ⁻²	Z3	1.5 × 10 ⁻²	A12*	1.2 × 10 ⁻³	R5A12	6.7 × 10 ⁻³
R7*	2.8 × 10 ⁻⁵	Z7	2.9 × 10 ⁻⁵	A5	1.0 × 10 ⁻⁵	R5A5	1.6 × 10 ⁻⁵
R6*	1.0 × 10 ⁻⁴			A6	2.6 × 10 ⁻⁵		
R1	1.3 × 10 ⁻⁴						
R9	4.6 × 10 ⁻⁵						
R10	2.1 × 10 ⁻⁵						
R2	6.9 × 10 ⁻⁶						
R4	4.0 × 10 ⁻⁴						

Wild-type double-stranded DNA molecules were completely digested with *HindII* (giving rise to *R* fragments, *AluI* (*A* fragments) and *HaeIII* (*Z* fragments)). Subsequently the fragments were fractionated with gel electrophoresis^{13,14}. *R5* fragment was completely digested further with the *AluI* enzyme. Subfragments *R5A5* and *R5A12* were used in the experiments. The fractionated double-stranded DNA fragments were annealed to *to8* single-stranded DNA⁷. These partial hybrid molecules were given to spheroplasts of *E. coli* K58 (*su*⁺) and after a 2–3-h growth period the yield was determined on *E. coli* HF4712 (total yield) and on *E. coli* C (wild type). The rationale behind the experiment is that if the *to8* mutation is covered by a fragment carrying the wild-type allele, the share of wild-type particles in the yield after spheroplast infection (that is, the ratio wild type-total) will be increased markedly^{7,15}. The fragments are ordered according to their sequence on the genetic map. The localisation with the *R* fragments showed *R5* as the fragment with the wild-type allele. So, of the *Z* and *A* fragments only the ones in the region of *R5* had to be tested.

*The *R6*, *R7* and *A12* preparations contain respectively 3, 2 and 4 different fragments of the same size.

fragment made from *R5* by *AluI* digestion). In this way we were able to insert *to8* in the genetic map (Fig. 1). The other mutants of this 'new cistron' also map on the *R5A12* fragment. It is clear that *to8* maps within gene *A*, and is separated from gene *B* by the *A* mutants *am35* and *am18*.

We next looked at the DNA synthesis in restrictive conditions in the presence and absence of chloramphenicol (CAM). With CAM only progeny RF DNA synthesis is permitted¹⁶; without CAM both RF and single-stranded DNA synthesis can proceed. As a control *am3* (gene *E*) and *am33* (gene *A*) were included in the experiment. Figure 2a shows that *to8* synthesises progeny RF DNA at the same rate as *am3*, a lysis-defective mutant not blocked in DNA replication¹⁶. Without CAM (Fig. 2b) the DNA synthesis of *to8* stops at a time when single-stranded DNA synthesis starts¹⁷. The gene *A* mutant *am33* shows no significant DNA synthesis in both experiments.

test, and all mapped on the *R5A12* fragment. Several 'real' gene *A* mutants were isolated, of which *amR8-1*, located at the *R8* fragment, is nearest to *to8* (Fig. 1). So the maximum size of the *to8* region is the maximum distance between *amR8-1* and *am35* (ref. 7), which is about 600 base pairs.

W. E. BORRIAS
P. J. WEISBEEK
G. A. VAN ARKEL

Department of Molecular Cell Biology
and Institute of Molecular Biology,
State University, Utrecht,
The Netherlands

Received February 25; accepted March 31, 1976.

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Expression of flagellar genes carried by bacteriophage lambda

THE specific interaction of many components is necessary for the assembly and function of flagella in *Escherichia coli*. Twenty genes have been shown to participate^{1–3} and at least eleven polypeptides are associated with the structure of the flagellar organelle⁴. The synthesis and assembly of these components and of flagellin, the major flagellar subunit protein, is tightly coupled and regulated^{5–7}. It has not been possible to analyse these regulatory mechanisms in great detail because: (1) the tight coupling between events usually leads either to the synthesis of a complete organelle, or to no detectable flagellar structure, and (2) most of the flagellar components are present at low levels and their synthesis is difficult to measure. To analyse the hierarchy of control and regulation in this system we have prepared a series of hybrid transducing phages that carry different combinations of flagellar genes. We describe here one of these phages and show that it can be used to study the expression of flagellar gene activities.

Hybrid lambda (λ) *E. coli* DNA molecules were constructed using λ gt- λ c DNA as the vehicle and isolated F factors as a source of DNA carrying flagellar genes. Figure 1 shows the results of *Eco*RI endonuclease digestion of two of these episomes. F 1338 carries about 2% of the *E. coli* chromosome (Fig. 1) and includes most of the genes that are involved with flagellar assembly and function. F 1906 is a deleted episome⁸ derived from F 1338; it lacks more than half the flagellar genes. Approximately 20 distinct bands can be distinguished in the digest of F 1906, and 33 bands in the digest of F 1338. The F 1338 digest was used to prepare hybrids according to the procedures described by Thomas *et al.*⁹. After transfection with hybrid molecules, a large number of λ plaques were picked and purified and lysates were prepared. The lysates were tested for their ability to transduce specific flagellar genes by using them to cross streak various λ lysogens on motility plates. Each lysogen carried a different flagellar gene mutation. Phages that carried specific flagellar genes were able to complement the appropriate lysogen which could then form trails in motility agar. Both abortive transduction and recombination could be scored on the motility plates. In this way various transducing phages have been isolated. We will describe one of these λ fla1.

Tests for abortive transduction showed that λ fla1 carried the genes *hag*, *fla*N and *fla*B. The *hag* gene controls the structure of flagellin, the major subunit component of the flagellar filament. *Fla*N and *fla*B refer to genes that include mutations resulting in bacterial strains that have no recognisable flagellar filaments. *Eco*RI endonuclease digestion and Agarose gel electrophoresis of λ fla1 DNA gave three bands. The relative mobility of the upper band suggested that it represented the left-hand fragment of λ gt- λ c. The second

band had a mobility which corresponded to the right-hand fragment of λ (1.1×10^7 daltons). The third band is barely resolved from the others; it corresponds to the incorporated piece of *E. coli* DNA. Its relative mobility suggests that it is one of the high molecular weight bands seen in the digest of F 1338, but not in F 1906.

To characterise further the incorporated DNA, a series of deletions of λ fla1 was prepared. Analysis by abortive transduction revealed the genetic functions that were deleted from each phage, and the Agarose gel electrophoresis patterns of *Eco*RI endonuclease digests showed the corresponding portion of DNA that was removed (Fig. 2). Deletions extending into the incorporated piece of DNA from the right-hand side can remove the *Eco*RI site and fuse the incorporated piece to the right-hand fragment, thus decreasing its mobility on electrophoresis. The same kind of argument applies to deletions entering from the left-hand side and from the middle of the incorporated DNA. The subsequent changes in the apparent molecular weight of the fragments facilitate determination of the relative orientation of the genes with respect to the two ends of λ (Fig. 2).

The deleted phages were used to study the expression of *E. coli* gene activity by measuring incorporation of amino acids into proteins synthesised in ultraviolet-irradiated λ lysogens. Essentially the same procedure was used as that

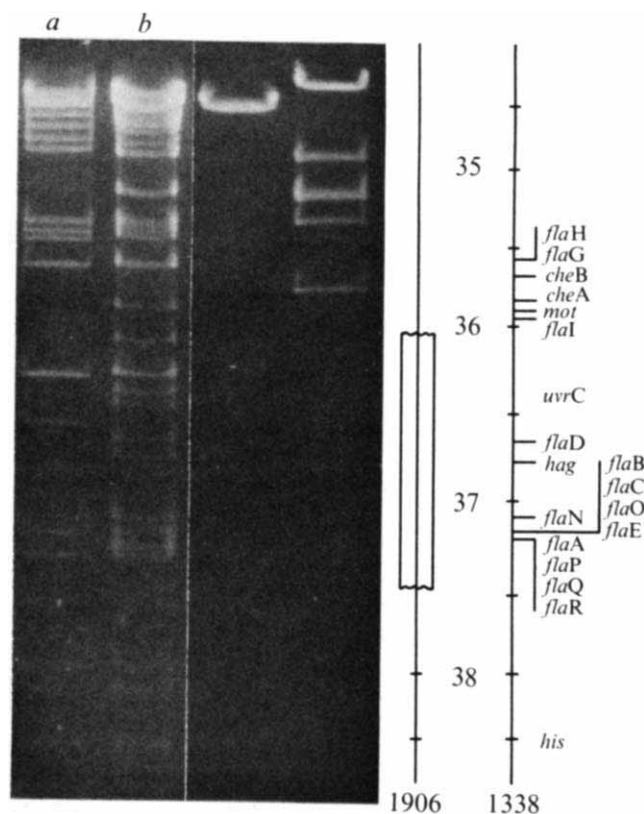


Fig. 1 Electrophoresis of *Eco*RI endonuclease digests of episomal DNA. The episomes were isolated by Sarkosyl lysis¹⁴. After an initial caesium chloride-ethidium bromide equilibrium centrifugation, the major portion of the chromosomal DNA was withdrawn without perturbing the band of episomal DNA. The solutions containing the episomal DNA were pooled and recentrifuged. Finally, the band corresponding to the density of covalently closed circular DNA was removed carefully. The DNA was precipitated¹⁵ and 2–4 μ g was treated with an excess of *Eco*RI endonuclease. After incubation with the enzyme, the samples were heated at 60 °C for 5 min and placed on 0.8% Agarose gel for electrophoresis. On the left is the pattern obtained from F 1906 DNA (a) and next to it the electrophoretogram of the digest of F 1338 DNA (b). For comparison, the *Eco*RI-treated plasmid (c) PML1 (9×10^6 daltons)¹⁶ and λ (d) DNA are shown. The map on the right shows the genetic loci carried by the episomes.

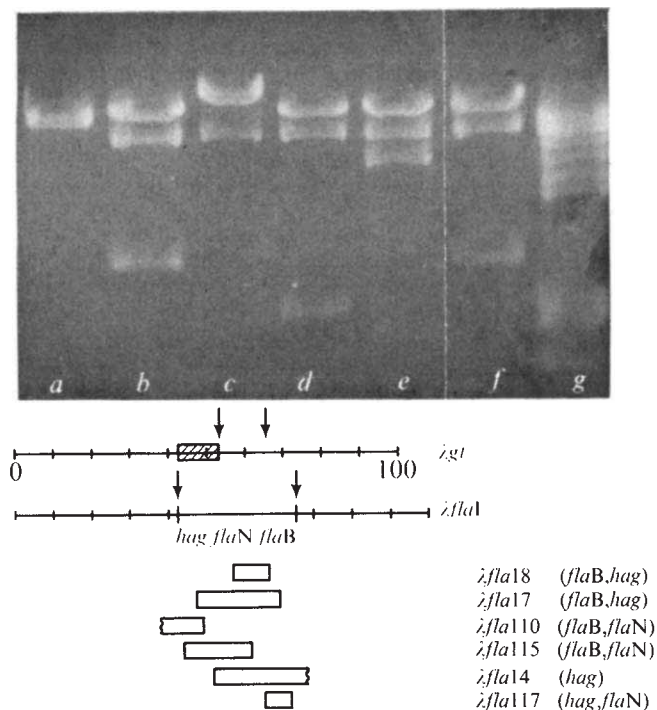


Fig. 2 Mapping of deletions in λ flal. The Agarose gel electrophoretogram shows the results of *EcoRI* digestion of DNA obtained from deletion mutants of λ flal. The deletions were selected by the pyrophosphate shock technique¹⁷. Surviving plaques were picked and lysates prepared. After genetic tests to determine the remaining markers, DNA was prepared from the phages by phenol extraction. The DNA was treated with *EcoRI* endonuclease and electrophoresis was on 0.8% Agarose gels. a, λ flal14; b, λ flal115; c, λ flal110; d, λ flal17; e, λ flal18; f, λ gt- λ c; g, F 1906. The map below is derived from the data in this and other electrophoretograms. The order of the *E. coli* genes is shown. The relative distribution corresponds to that seen on the map in Fig. 1: *flaN* is close to *flaB* and further away from *hag*.

described by Jaskunas *et al.*¹⁰⁻¹². Figure 3 shows that a major band consistently appears in the acrylamide gel electrophoresis patterns of extracts of cells infected with λ s carrying the *hag* gene. Phages that do not carry *hag* but carry either the *flaB* or the *flaN* gene, or both, do not show

this band. The band has a mobility which corresponds to an apparent molecular weight of 54,000, and it seems to have two minor bands associated with it, one at about 56,000 and another at 60,000. Furthermore, as the extract is heated at 100 °C for 20 min almost all the material shows a mobility corresponding to a molecular weight of 60,000. This pattern is characteristic of *E. coli* flagellin¹³. The evidence that this band corresponds to flagellin can be summarised as follows. (1) It comigrates precisely with authentic flagellin on one-dimensional acrylamide gels and shows the same characteristic band pattern. (2) It comigrates precisely on two-dimensional acrylamide gels prepared by the techniques described by O'Farrell¹⁴, with authentic *E. coli* flagellin. (3) It only appears in extracts of cells infected with phages that have been shown by genetic techniques to carry the *hag* gene, which is the structural gene for flagellin. (4) Material forming the band which corresponds to flagellin is precipitated by anti-flagellar antibody and removed from the cells by shearing.

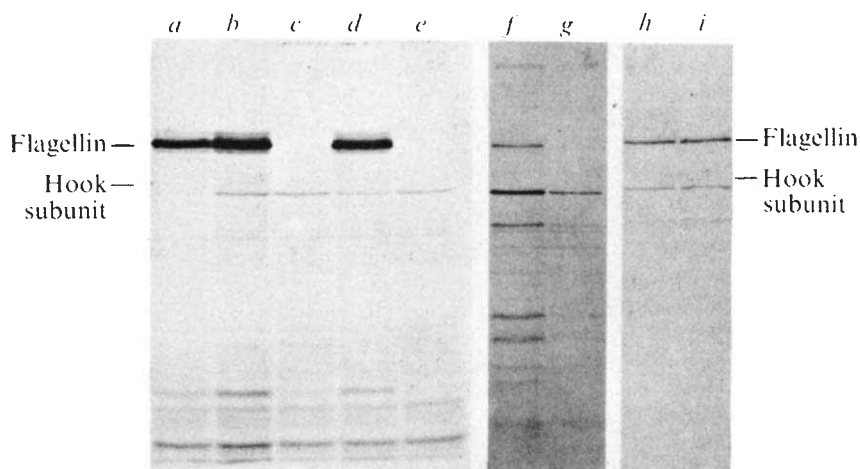
To test the regulation of flagellin synthesis, several mutant strains carrying flagellar gene mutations were prepared. Figure 3 shows the results obtained from infections of *hag*, *mot* and *flaI* mutants by λ flal. Clearly flagellin is synthesised in the host strains which carry *hag* (Fig. 3f and i) or *mot* (Fig. 3h) mutations but it is not synthesised in the host which carries the *flaI* (Fig. 3g) mutation. Earlier experiments with flagellar mutants⁷ suggested that the *flaI* gene product is necessary for *hag* gene expression. This system may be useful for studying the interactions between the *flaI* gene product and the *hag* gene.

We have not so far defined bands on acrylamide gels which correspond to products of the *flaB* or *flaN* genes. If these genes specify structural components of the flagellar basal structure, they may be read at rates that are 100 to 1,000 times slower than flagellin; this could account for our not having observed them. It should be possible, however, to prepare phages where these genes are oriented so that their expression can be affected by the leftward λ promoter. Jaskunas *et al.*¹² have shown that such an arrangement leads to synthesis of *E. coli* gene products at relative rates determined by the λ promoter.

The hybrid phages seem to make available a new way of looking at the regulation of synthesis, assembly and function of the bacterial flagellar organelle.

We thank Jeff Velten and John Abelson for help, Vickers

Fig. 3 Electrophoresis of proteins synthesised in ultraviolet-irradiated bacteria infected with hybrid λ phage. The infection of ultraviolet-irradiated cells was performed exactly as described by Jaskunas *et al.*^{10,11}. After the infected, irradiated cells were labelled, they were pelleted and resuspended in sodium dodecyl sulphate (SDS)-gel sample buffer (3% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.06 M Tris-HCl, pH 6.8), heated for 1 min at 100 °C, and electrophoresed. Radioactively labelled marker proteins were used and the samples were loaded on to a 12.5% polyacrylamide gel slab containing 0.1% SDS. After electrophoresis the gels were dried and subjected to autoradiography. The host strain was either *E. coli* K12 strain 159 carrying λ (obtained from Dr H. Murialdo)¹⁸, or mutants derived from this strain by mutagenesis and selection with bacteriophage chi. The chi-resistant mutants derived from 159 λ were screened by complementation and strains carrying *hag* (MS6101), *mot* (6106) and *flaI* (6103) mutations were chosen. Samples a, b, c, d, and e show the results of infection of 159 λ with: a, λ flal14 (*hag*); b, λ flal18 (*flaB*, *hag*); c, λ flal115 (*flaB*, *flaN*); d, λ flal117 (*hag*, *flaN*); e, λ flal110 (*flaB*, *flaN*). These autoradiograms were incubated with film for 5 d. The strains carrying flagellar mutations were infected



with λ flal at a multiplicity of approximately 10. f, *hag* (MS6101), a clear band corresponding to flagellin is seen. g, *flaI* (MS6103), no apparent flagellin material was found. h, *mot* (MS6106), a flagellin band appeared. i,

hag (MS6101) was infected with λ flal14—another λ phage carrying the flagellin gene at a multiplicity of approximately 10. These autoradiograms were incubated with film for 1 d.

Hershfield for advice and Rick Firtel for *EcoRI* endonuclease. The work was supported by a grant from the NSF.

MICHAEL SILERMAN
PHILIP MATSUMURA
ROCKFORD DRAPER
STEVEN EDWARDS
MELVIN I. SIMON

University of California, San Diego,
Department of Biology,
La Jolla, California 90293

Received January 29; accepted March 23, 1976.

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“Non-rigid” nucleotides in tRNA: a new correlation in the conformation of a ribose

WE have reported the three-dimensional structure of yeast tRNA^{Phe}, determined by fitting a skeletal wire model to an electron density map at a resolution of 2.5 Å, phased by the method of isomorphous replacement¹. Atomic coordinates have been published for the unrefined structure². We are in the process of refining our structure using difference Fourier techniques together with the real-space refinement procedure of Diamond³. The crystallographic residual (*R*) at 2.5 Å is 0.31, a value comparable to that found for proteins at a similar stage.

On the basis of a survey of the known nucleotide and nucleoside structures, several groups have suggested that the conformational angles are naturally confined to a few narrow ranges^{4,5}, and this concept has been embodied in the term “rigid nucleotide”. The essence of this idea is that although a single nucleotide unit in a polymer or oligomer has six main-chain dihedral angles, the two on either side of the phosphorus atom (ω and ω') are the only ones which are free to vary, the others being confined to a narrow range. By applying this concept to folded nucleic acids, attempts have been made to predict the structures of non-helical regions^{5,6}. Refinement of the tRNA structure tests to what extent these ideas and predictions are correct. We have found that the polynucleotide chain is able to explore many more conformations than are implied by the rigid nucleotide hypothesis, and thus that most previous energy calculations (for example ref. 7) and structural speculations have too limited a basis.

While we were building the wire model to fit the isomorphous replacement density map, it became clear that some ribose rings had to adopt the C2'-endo conformation (in contrast to the more usual C3'-endo found in RNA double helices), to fit the density for the pendant base and the phosphate groups on either side. This change in the sugar pucker implies a change of the dihedral angle ψ (Fig. 1) from 83° to 146°, and thus has a considerable effect on the course of the chain. Most of the loops and extended regions in tRNA involve several such unusual sugars.

As well as changing the sugar pucker, we were obliged, in fitting the map, to change the conformation at the C4'-C5' bond in a number of places. The normal conformation here (Fig. 1a) is the so-called *gauche*⁺ (*g*⁺, also called *gg*) in which the angle ψ (O5'-C5'-C4'-C3') is +60°. (The sign convention is that, looking along the C5'-C4' bond, a rotation of the nearest bond clockwise by 60° leads to an eclipsed conformation.) In addition to a number of residues in which conformation about the C5'-C4' bond, is *trans* (*t* or *gt*), with $\psi=180^\circ$, we found four instances in which the value of ψ is -60° (the rare *g*⁻, or *tg*, conformation). In all four of these cases, the ribose ring has the C2'-endo pucker. The reason for this correlation is clear: in a C3'-endo ribose with $\psi=-60^\circ$ (*g*⁻), the distance between O3' and O5' would be only 3.04 Å. By changing the ring pucker to C2'-endo, this unfavourable electrostatic interaction is relieved, the O3'-O5' distance increasing to 3.96 Å (Fig. 2).

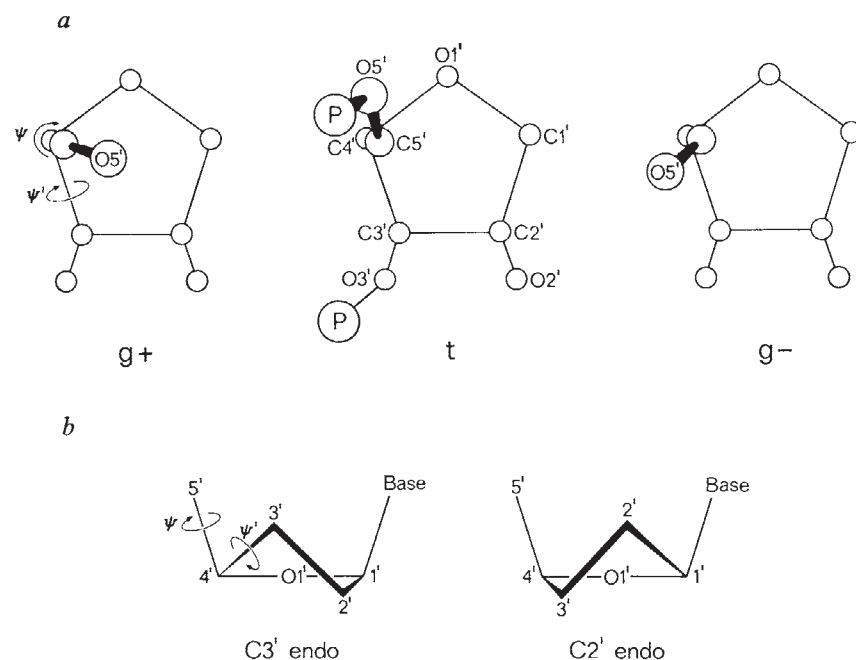


Fig. 1 Schematic diagrams of the ribose ring showing the approximate conformations at (a) the C4'-C5' bond, torsion angle ψ , and (b) the C3'-C4' bond, torsion angle ψ' . In a the ribose is viewed face on, and in b edge on with the pucker exaggerated.

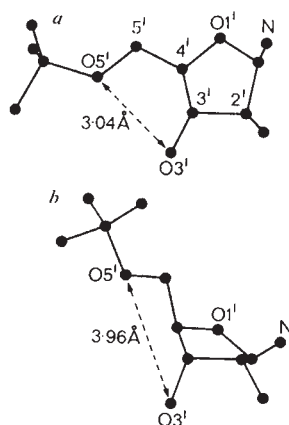


Fig. 2 Projection of a nucleotide on to the plane O3'-C3'-C2' in the two conformations g^- , C3'-endo (a) and g^- , C2'-endo (b). Most of the base has been omitted for clarity.

The t conformation at C5'-C4' is quite common in nucleosides and 3'-nucleotides and may occur in "kinked" DNA double helices⁸. In tRNA, we believe it occurs at the riboses on either side of the guanine residue involved in the G-U "wobble" base pair¹. The g^- conformation, however, is much rarer, having been found in only seven nucleoside crystal structures^{3,9}. On inspection of these structures, we find, in agreement with our results on tRNA, that in all seven, in which nine crystallographically independent molecules are represented, all the ribose rings have either the C2'-endo or the closely related C1'-exo conformation. In one of the cases (5'-bromo-5'-dA)¹⁰, the O5' atom is replaced by bromine; in all of the others for which full details are available, the O5' can form intermolecular hydrogen bonds, and this presumably provides the driving force towards the unusual structure. Although no known nucleotides have the g^- conformation, the further interactions occurring in polynucleotides (for example base-stacking and intercalation) may sometimes favour the unusual staggered forms.

It is clear that loop formation in folded nucleic acids is not as straightforward as hitherto supposed; although all the ω - ω' pairs in our refined tRNA structure fall in energetically favourable regions of the ω - ω' plot⁷, we must take into account the two extra factors of sugar pucker and rotation about C5'-C4' when considering loop formation. The anticodon loop is the only example in which the folding is achieved by varying only ω and ω' .

Coordinates for the same tRNA molecule in a different crystal form have been published recently by another group¹¹. These coordinates are based on a refinement against X-ray data at 2.7-Å resolution. In contrast to our results, their ψ values show all the ribose groups to have the g^+ conformation about C5'-C4'. A second set of coordinates for the orthorhombic crystal form¹² does contain some sugars in conformations other than g^+ , but generally not in the same places as we find. Although we cannot yet be certain that we have all our ribose conformations correct, most of them have been sustained during refinement. The correlation we have found between g^- and C2'-endo, with its simple physical explanation, argues strongly in favour of our interpretation.

A. JACK
A. KLUG
J. E. LADNER

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge CB2 2QH, UK

Received March 12; accepted April 1, 1976.

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Production of nerve growth factor by the mouse adrenal medulla

PREVIOUS studies in our laboratories¹ have demonstrated that nerve fibres from explanted chick embryonic sensory ganglia become preferentially orientated towards fragments of mouse sarcoma 180 or adrenal medulla. It was suggested that this orientation was mediated by the release from the tissues of nerve growth factor (NGF). Direct experimental evidence for this hypothesis was not presented but the effect could be duplicated by using a capillary tube containing a solution of purified NGF instead of the tissue fragment, thus establishing that the protein could exert a directional effect on the growth of nerve fibres *in vitro*. Independently, Burnstock and coworkers² have reported similar orientation effects for rat sympathetic ganglia grown in proximity to a range of rat and other tissues. These results are potentially of considerable importance since they suggest that the orientation of peripheral nerves *in vivo* may be regulated, at least in part, by the release of NGF from the end organ. We report here the results of further experiments which confirm that NGF, as identified by biological activity and specific immunological properties, is produced and released by the adrenal medulla in culture.

Table 1 Orientation of nerve fibres towards and away from explants of adrenal medulla in tissue culture

Score	Towards adrenal	Away from adrenal
0	54	133
0.5	57	16
1	19	3
1.5	16	2
2	6	0
2.5	2	0

Frequencies with which the stated scores (on the accepted arbitrary scale) were obtained. From these data the mean scores $\pm$ s.e.m. are 0.57 ± 0.05 (towards) and 0.09 ± 0.02 (away). The frequency distributions are significantly different ($\chi^2 = 179$, $P < 0.001$). For experimental details, see text.

The orientation of nerve fibres promoted by the mouse adrenal medulla is evident from the results given in Table 1. Cultures were set up essentially as previously described¹ and the fibre outgrowth assessed on the accepted arbitrary scale¹. The effect was investigated further by maintaining intact adrenal glands (4-5 per preparation, 578 total) from fully mature male mice (Swiss albinos) as organ cultures essentially by the method of Trowell⁴. The culture medium was changed at the half-way stage of the experiment (24 h) and the total medium collected and pooled. Parallel control experiments without tissue were carried out simultaneously. The recovered media were then concentrated (~20 times) by ultrafiltration (Minicon concentrators, 15,000 MW cutoff, Amicon). The adrenal glands were recovered at the end of the experiment and the water-soluble material was extracted and lyophilised. A comparable extract from freshly dissected glands (546 total) was also made. The samples were then tested for NGF activity in the standard bioassay^{5,6}. The adrenal glands were found

to contain no detectable activity before the organ culture but released biologically active material into the culture medium and thereafter contained significant levels of the factor. (Tables 2 and 3.)

The immunological properties of the active material were then examined to establish that the observed effects were due specifically to NGF and not to some other, general growth-promoting agent. Two main types of NGF have been isolated; those from mouse salivary gland and from snake venom^{7,8}. The factors are immunologically distinct^{9,10} as shown by different titres with homologous and heterologous antisera (see also Table 4). NGF of the immunological type found in snake venom is not confined to this species but occurs also in mammalian sera⁹. The active material from the mouse adrenal medulla was now found to have identical immunological properties (within the limitations of the test) to the NGF from mouse salivary gland (Table 4). Further, the orientation effect described above was blocked by similarly high dilutions of the antiserum to the salivary protein (Table 4).

Table 2 NGF activity in concentrated organ culture media from preparations of adrenal medulla

Score	Experimental	Control
0	12	79
0.5	34	3
1	22	0
1.5	7	0
2	1	0

Cultures were maintained in a water-saturated atmosphere of 95% oxygen and 5% carbon dioxide in a pre-selected medium (1–1.5 ml per preparation, all components from Gibco-Biocult) consisting of RPMI 1640 (80%), foetal calf serum (10%), tryptose phosphate broth (10%), penicillin (100 units ml⁻¹), streptomycin (100 µg ml⁻¹) and L-glutamine (2 mM). The table gives the frequencies with which the stated scores (on the accepted arbitrary scale) were obtained for the concentrated media cultured with (experimental) and without (control) tissue. From these data the mean scores $\pm$ s.e.m. are 0.68 ± 0.05 (experimental) and 0.02 ± 0.01 (control). The frequency distributions are significantly different ($\chi^2 = 432$, $P < 0.001$). For further details, see text.

These results do not, however, distinguish between the *de novo* synthesis of NGF in culture and the activation and release from the gland of a potential precursor. Such an activation might be achieved by proteolytic cleavage, possibly resulting from the action of a native esterase as has been tentatively suggested for the NGF from mouse salivary gland¹¹. To test this hypothesis, a soluble extract of mouse adrenal glands (470 glands, 15% w/v in water) was incubated (37 °C, 24 h) in the presence and absence of the

Table 3 NGF activity in soluble extracts of adrenal medulla before and after culture

Score	Cultured adrenals	Fresh adrenals
0	0	20
0.5	9	1
1	4	0
1.5	5	0
2	2	0

Glands (cultured or fresh) were homogenised in distilled water (30% w/v) and the extract centrifuged (48,000g, 60 min) and lyophilised. In general 1g wet weight of tissue gave ~15 mg of lyophilised extract. Samples were assayed at a final concentration of 5 mg ml⁻¹: the table gives the frequencies with which the stated scores (on the accepted arbitrary scale) were obtained. From these data the mean scores $\pm$ s.e.m. are 1.00 ± 0.12 (cultured) and 0.02 ± 0.02 (fresh). The frequency distributions are significantly different ($\chi^2 = 27$, $P < 0.001$). In addition, preparations of microsomes (both before and after treatment with 1% Triton X-100 to liberate any membrane-bound protein) from uncultured adrenals were inactive. For further details, see text.

purified γ (esteropeptidase) subunit from the mouse 7S NGF (ref. 12) and some of the esterase-active and other fractions from the purification of the latter protein¹³. In no case was NGF activity detectable (at 0.5 mg ml⁻¹ lyophilised extract) in bioassay. A further possibility, apparent in all studies on the biosynthesis of NGF *in vitro*, is that the tissue releases an enzyme which causes the formation of NGF from a precursor in the culture medium. This precursor would presumably be located in the serum component of the medium. The adrenal clearly does not normally contain such an enzyme, however, since the standard bioassay of the soluble extract (which gives negative results) involves incubation of the material in a tissue culture medium essentially similar to that used in the organ culture experiments. More importantly, the NGF detected in the present study is immunologically identical to the protein from mouse salivary gland and is therefore more likely to be derived from the mouse adrenal medulla than from foetal calf serum. We have shown previously⁹ that the growth factors present in human and mouse sera are immunologically distinct from the salivary protein and Oger *et al.*¹⁴ have reported that foetal calf serum does not contain immunoreactive NGF as judged by a highly sensitive phage assay based on this protein.

Table 4 Immunological properties of the NGF from mouse adrenal medulla

Antigen	Mouse submaxillary gland NGF	Antibody <i>Vipera russelli</i> NGF
Mouse submaxillary gland NGF (purified)	6,000	15
<i>Vipera russelli</i> NGF (purified)	< 3	6,000
Adrenal medulla, orientation effect	3,000	15
Adrenal medulla, concentrated organ culture medium	6,000	15

The maximum dilution of the appropriate antiserum which will block the effect of 1 BU of the NGF preparation (on the accepted arbitrary scale) or completely inhibit the production of nerve fibres in orientation experiments.

Our results then suggest that the level of NGF in the normal adrenal is < 1 biological unit (BU) per g wet weight, the limit detectable by bioassay under the conditions used. This finding is in sharp contrast to those of Hendry¹⁵ and Johnson *et al.*¹⁶ who obtained by radioimmunoassay values of 100–300 BU g⁻¹. To reduce the possibility of interference in the bioassay, a soluble extract (200 glands) was fractionated by ion exchange chromatography as previously reported for mammalian sera⁹ but again no activity could be detected. A possible explanation of these differences is that the adrenal contains NGF in a form which is biologically inactive but capable of combining with antibody. To test this hypothesis, the homologous antiserum titre for salivary NGF was determined in the presence and absence of adrenal extract (0.5 mg ml⁻¹). The titres were the same in both cases, however, showing that the extract did not contain significant amounts of material capable of competing with NGF for antibody. According to the data from radioimmunoassay, a change in titre of up to tenfold would have been expected. These findings may reflect the reported difficulty¹⁷ in obtaining NGF in sufficiently highly purified form to permit valid immunochemical studies. This is particularly relevant for studies based on radioimmunoassay, which is an extremely sensitive procedure.

There have been a number of reports on the synthesis *in vitro* of NGF by cell lines and tissues^{14,18-22}. We believe, however, that we are the first to demonstrate the production of NGF *in vitro* by an organ that does not normally contain the protein *in vivo*. Our findings may relate to those of Hendry and Iversen²³ who showed that after sialectomy in the mouse, which apparently removes the major site of synthesis of NGF in the animal, other unidentified organs begin to secrete the factor. The adrenal gland may be such an organ. Alternatively, our findings may imply that the adrenal begins to synthesise NGF in culture in response to the artificial conditions of the experiment. In this event, any attempt to extrapolate from the production of NGF *in vitro* to the situation *in vivo* must be made with great caution. This may be particularly relevant since preliminary studies show that the synthesis of NGF in culture is not restricted to the adrenal medulla but may be a general property of mouse and rat tissues.

We thank the Science Research Council for the award of a Postgraduate Studentship to G.P.H., Dr D. C. Edwards (Wellcome Research Laboratories) for the gifts of antisera and mice and Dr S. J. Walter (Charing Cross Hospital Medical School) for providing the fractions from mouse salivary gland.

G. P. HARPER
F. L. PEARCE
C. A. VERNON

The Christopher Ingold Laboratories,
Department of Chemistry,
University College London,
London WC1H 0AJ, UK

Received December 15, 1975; accepted March 25, 1976.

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head (Fig. 1). The pathways from the retina to the visual cortex undergo partial decussation in the chiasma, so that information presented to the left half of the visual field passes to the right hemisphere, whereas the left hemisphere receives signals from the right half of the visual field. Consequently, it might be predicted that stimulation of one half field will produce an evoked response which is maximal over the contralateral hemisphere. The data presented here show that, contrary to this prediction, the maximal response occurs at the scalp electrodes situated over the hemisphere ipsilateral to the field stimulated.

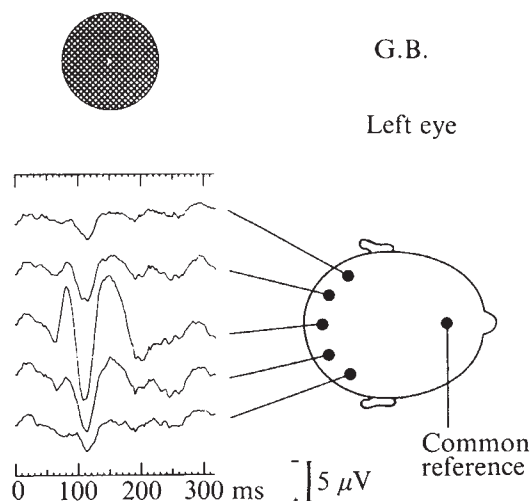


Fig. 1 Monopolar recording of the response to stimulation of the whole field (out to 16°) for the left eye of subject G.B. The positive large component at about 110 ms is seen maximally at the midline electrode and falls off fairly symmetrically in amplitude on the two sides of the head. Time scale in 10, 50 and 100 ms marks.

The average evoked response to stimulation of the left and right half fields for each eye of nine healthy individuals (age range 19-29 yr) was recorded from a chain of chlorided silver cup electrodes 5 cm up from theinion. The central electrode of the chain was in the midline and the other electrodes were spaced 5 cm and 10 cm laterally on either side. Each electrode was referred to a common midfrontal electrode 12 cm above the nasion and in the midline. A black-and-white checkerboard pattern was back-projected on to a translucent screen which was masked to produce a semicircular field extending 16° out from the fixation point for a subject sitting 1 m from the screen. The subject was instructed to fixate a constantly illuminated spot of light at the centre of the vertical diameter of the stimulus field. Each square of the checkerboard subtended an angle of 50' and the brightness of the white squares as seen by the subject was 227 cd m⁻², whereas that of the black squares was 8.2 cd m⁻². The checkerboard pattern was reversed twice per second and the electrical activity at the five electrodes was sampled at 400 Hz for 320 ms after each reversal. The average evoked response for 200 complementary pattern reversals was recorded with a PDP-12 computer.

The monopolar records in Fig. 2 show that, paradoxically, the response to stimulation of a half field is recorded maximally from the midline and from electrodes over the hemisphere ipsilateral to the field of stimulation, whereas the response on the contralateral side is comparatively flat. This ipsilateral positivity was recorded consistently in every subject for each half field and for stimulation of either eye. The bipolar records in Fig. 2 show the distribution of responses recorded with the five occipital electrodes connected as a bipolar chain rather than linked to a common midfrontal reference; the maximal response is now apparently recorded over the contralateral hemisphere. Comparison of

A paradox in the lateralisation of the visual evoked response

THE average evoked response in man to stimulation of the visual field with a reversing checkerboard pattern and recorded from the scalp over the occipital region has a waveform which is consistent both within and between subjects. The most characteristic feature for full-field stimulation is a major positive component at about 100 ms which is recorded maximally 5 cm above theinion in the midline and is distributed fairly symmetrically over both sides of the

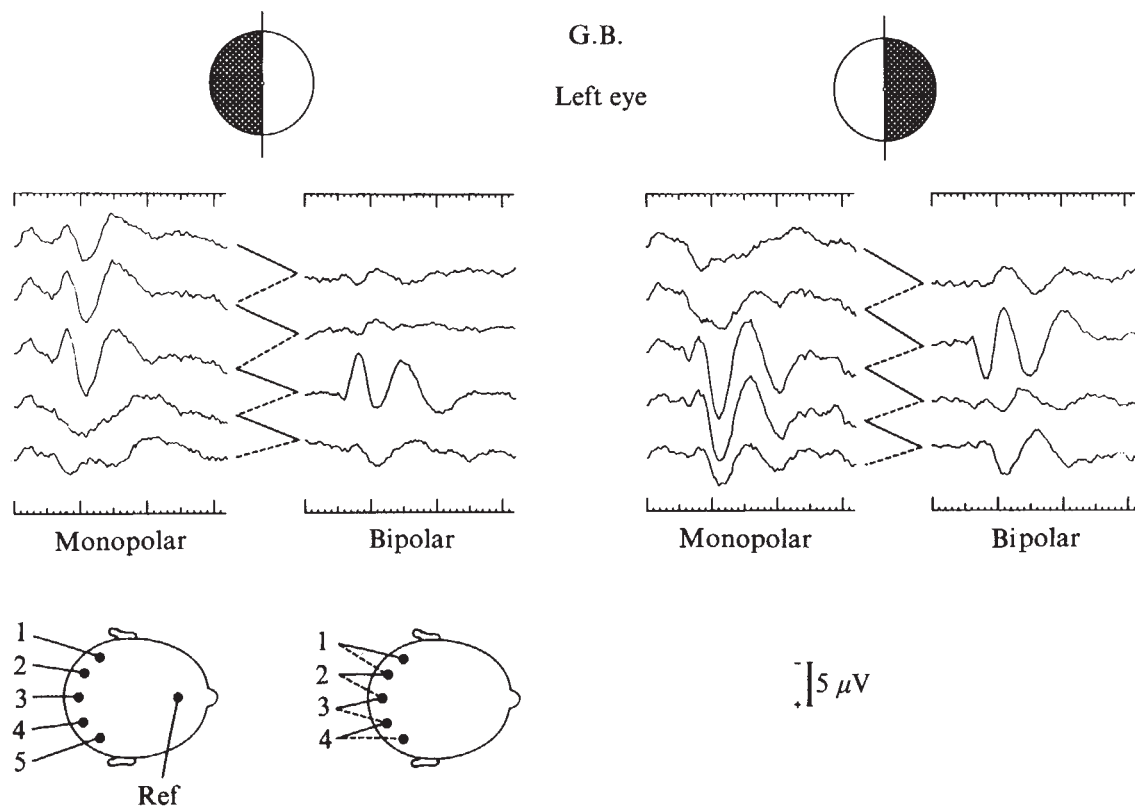


Fig. 2 Monopolar and bipolar records for stimulation of the two half fields for the left eye of subject G.B. In the monopolar record the large positive component at about 110 ms is seen at the midline electrode and at the electrodes ipsilateral to the stimulated half field, whereas the contralateral channels are relatively flat. In the bipolar record, which reflects voltage gradient, the ipsilateral channels show little or no response, whereas the midline–contralateral channel shows the maximum response.

the monopolar and bipolar records, however, shows that it is erroneous to conclude from such a bipolar record that the response is largest contralaterally; although the maximum voltage gradient is indeed between the midline electrode and its neighbour over the contralateral hemisphere, it is the response at the midline electrode that has the greater amplitude, as the monopolar record shows. The bipolar recording shows a comparatively flat response on the ipsilateral side, not because there is little or no activity, but because there is equally high amplitude activity at all

electrodes on this side of the scalp. Similarly, on the contralateral side, there is low amplitude activity at both electrodes, not the high amplitude activity that would be expected if the maximal response occurred over this hemisphere.

A possible explanation of this unexpected lateralisation of the evoked response is that the cortical generator areas for the pattern stimulus are largely situated on the medial and posteromedial surface of the visual cortex where the neurones are transversely oriented. Thus, electrodes over the hemisphere ipsilateral to the field stimulated are optimally placed to record the response from these generator areas, whereas electrodes over the contralateral hemisphere are not so placed. The schematic representation of this hypothesis in Fig. 3 shows that the electrodes on the left side of the head are best placed to record a response from the medial surface of the right visual cortex evoked by pattern stimulation of the left half field. The electrodes over the right hemisphere, however, are approximately perpendicular to the axis of the generator area and therefore record very little response.

The paradoxical distribution of the evoked response to stimulation of the visual half fields has important implications for the interpretation of recordings made in cases of hemianopia. The findings in healthy subjects can be used to predict the type of response asymmetry to be expected in cases of either homonymous or bitemporal hemianopia. In homonymous hemianopia the asymmetry of the evoked response recorded to whole-field pattern stimulation is the same for both eyes ('uncrossed' asymmetry) and the 'blind' field will be contralateral to the side over which the response is maximally recorded. In bitemporal hemianopia (that is, where the fibres from the nasal retina of each eye are damaged) the asymmetry is opposite for the two eyes ('crossed' asymmetry) and the maximal response will be

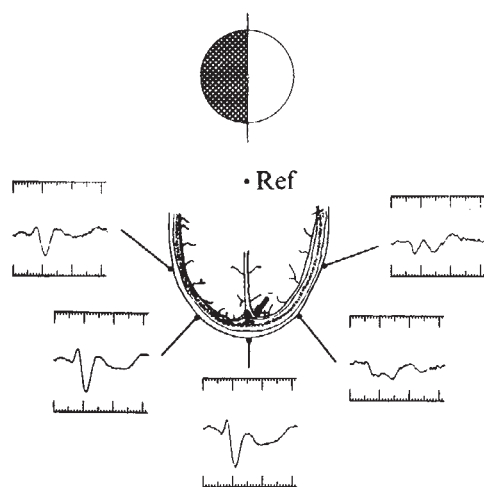


Fig. 3 Schematic representation of the relationship between the cortical generator area for the left half field response and the scalp electrodes. The electrodes at the midline and ipsilateral to the stimulated half field are best placed to record the response from the posteromedial surface of the contralateral visual cortex.

seen on the right side for the left eye and on the left side for the right eye. Recordings made from patients with hemianopia support these predictions¹.

In the present experiments a half field stimulus extending out to an eccentricity of 16° has been used, but a similar ipsilateral distribution of the major positive component of the pattern reversal response can also be recorded when stimulating smaller segments of each half field. It is still clearly demonstrable when only the central 0–4° are stimulated. When stimulation is confined to the macular area itself (0–2°), however, the response becomes less well lateralised and may even have a contralateral predominance. This is what would be predicted on the scheme illustrated in Fig. 3, as the cortical representation of the macula is at the posterior pole of the occipital lobe and the generator neurones are here facing posteriorly rather than medially.

G. BARRETT
L. BLUMHARDT
A. M. HALLIDAY
ELISE HALLIDAY
A. KRISS

Medical Research Council,
Institute of Neurology,
National Hospital,
Queen Square,
London WC1N 3BG, UK

Received January 26; accepted March 30, 1976.

¹ Halliday, A. M., Halliday, Elise, Kriss, A., McDonald, W. I., and Mushin, Joan, *Brain*, 99, 357–374 (1976).

Synaptic vesicle recycling in synaptosomes *in vitro*

PINCHED-OFF presynaptic nerve terminals (synaptosomes) prepared from rat brain homogenates^{1,2} retain many of the functional properties of intact neurones^{3,4}. They can accumulate K⁺ and extrude Na⁺ against concentration gradients^{5,6}, and their membranes seem to develop K⁺ diffusion potentials which behave like the membrane potentials of most neurones⁷. Furthermore, the isolated terminals exhibit an increased rate of Ca²⁺ accumulation⁸ and Ca²⁺-dependent transmitter release^{8–10} when exposed to depolarising agents such as veratridine or an increased external K⁺ concentration ([K]_o). The latter findings are consistent with the 'calcium hypothesis' of depolarisation–secretion coupling¹¹. The calcium which enters the terminals during depolarisation seems to trigger the release of transmitter from intraterminal vesicles by an exocytotic process involving transient fusion of the vesicle membrane with the surface membrane^{12–14}. This hypothesis is supported by experiments with electron-opaque extracellular markers; the appearance of the markers within intraterminal vesicles, after stimulation, has been taken as evidence for vesicle recycling^{15–17}. Here we report similar observations on synaptosomes incubated *in vitro*, showing that synaptosomes function like intact terminals in this respect as well.

Synaptosomes were prepared from whole rat brain homogenates by a slightly modified¹⁸ Gray and Whittaker procedure². The standard ('Na+5K') synaptosome incubation solution contained (mmol l⁻¹): NaCl, 145; KCl, 5; CaCl₂, 1.2; MgCl₂, 1.3; NaH₂PO₄, 1.2; glucose, 10; HEPES, 20; the solution was titrated to pH 7.6 at 20 °C with Tris base. In some instances, part of the NaCl was replaced mol-for-mol by KCl (= 'K-rich' incubation media; see below).

Synaptosomes from the sucrose gradient² were equilibrated⁷ with ice-cold Na+5K, and centrifuged at 9,000g

for 5 min at 4 °C. The supernatant was discarded, and the pellets resuspended in Na+5K (approximately 2 mg synaptosome protein ml⁻¹) and incubated for 12 min at 30 °C. Additional aliquots of Na+5K or of K-rich solution (to give a final [K]_o of 35 or 50 mM) were then added. In most experiments the incubation solutions also contained sufficient colloidal thorium dioxide (Thorotrast, a 25% suspension, from Fellows Testagar, Anaheim, California,

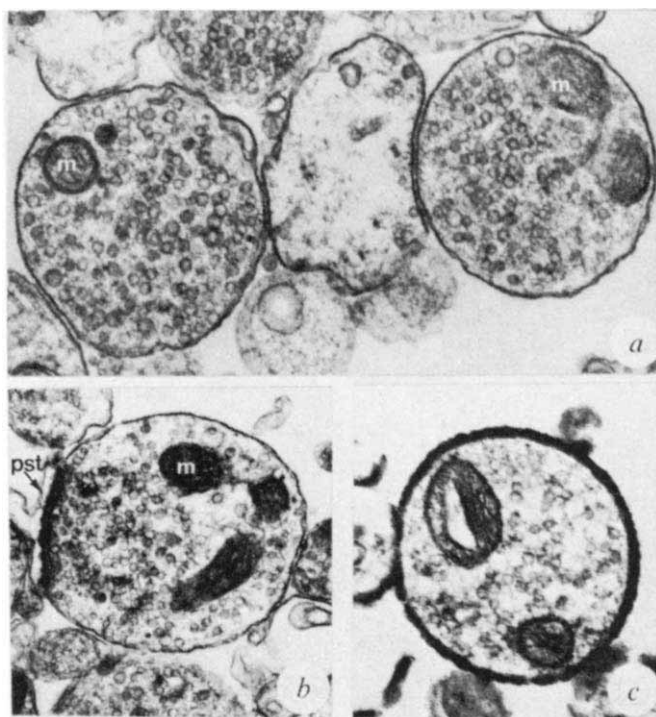


Fig. 1 Morphology of synaptosomes incubated in standard Na+5K. *a*, Vesicle-filled terminals with intraterminal mitochondria (m). No extracellular marker present. $\times 43,000$. *b*, Similar to *a*, with a clearly demarcated postsynaptic thickening (pst). $\times 33,000$. *c*, Synaptosome fixed and stained after 30 min incubation in Na+5K containing 5 mg ml⁻¹ HRP. Note halo of HRP reaction product around synaptosome, and absence of product in the cytoplasm. $\times 39,000$.

a gift from Dr A. Enders) to give a final concentration of 3.3%, or horseradish peroxidase (HRP, type VI, Sigma, St Louis, Missouri, previously dialysed¹⁵ against Na+5K) to give a final concentration of 5 mg ml⁻¹ after dilution.

The diluted suspensions were incubated at 30 °C for an additional 2–30 min. Aliquots were then diluted with one volume Na+5K plus two volumes Karnovsky's cacodylate-buffered fixative¹⁹, and refrigerated overnight; the samples were then centrifuged, and the supernatant solutions discarded. The HRP-treated pellets were reacted with H₂O₂ and diaminobenzidine¹⁹ for 2 h at room temperature, and washed several times in cacodylate buffer¹⁶. All pellets were stained with potassium ferrocyanide-reduced osmium tetroxide²⁰, followed by osmium tetroxide. They were dehydrated in increasing concentrations of ethanol and embedded in Araldite. Thin sections were examined electron microscopically.

Most synaptosomes appeared more-or-less circular in cross section (Figs 1 and 2). Those fixed after incubation in Na+5K were generally filled with uniform-sized vesicles (about 400–500 Å in diameter; Fig. 1*a–c*), although occasional larger vesicles were seen. The terminals also usually contained mitochondria (Fig. 1). When exposed to HRP, the terminals incubated in Na+5K were surrounded by a halo of HRP reaction product (Fig. 1*c*); the apparent lack of HRP penetration into the cytoplasm implies that the

surface membrane had resealed. In some terminals (see Fig. 2c), however, the extracellular marker was distributed diffusely throughout the cytoplasm, but was excluded from the synaptic vesicles; this presumably occurred in terminals which had not completely resealed, or which were subsequently damaged. The morphological markers may therefore be useful for the quantitative assessment of synaptosome integrity.

After exposure to media containing 35 or 50 mM K, the intraterminal vesicles appeared much more variable in size, and large vacuoles (cisternae¹⁶) were found in many terminals (Fig. 2a and c). Coated vesicles^{16,21} were observed in some of the terminals exposed to elevated [K]_o (Fig. 2b). Even after prolonged (15–30 min) incubations in K-rich media, the terminals were not markedly depleted of vesicles.

When incubated in HRP-containing K-rich media,

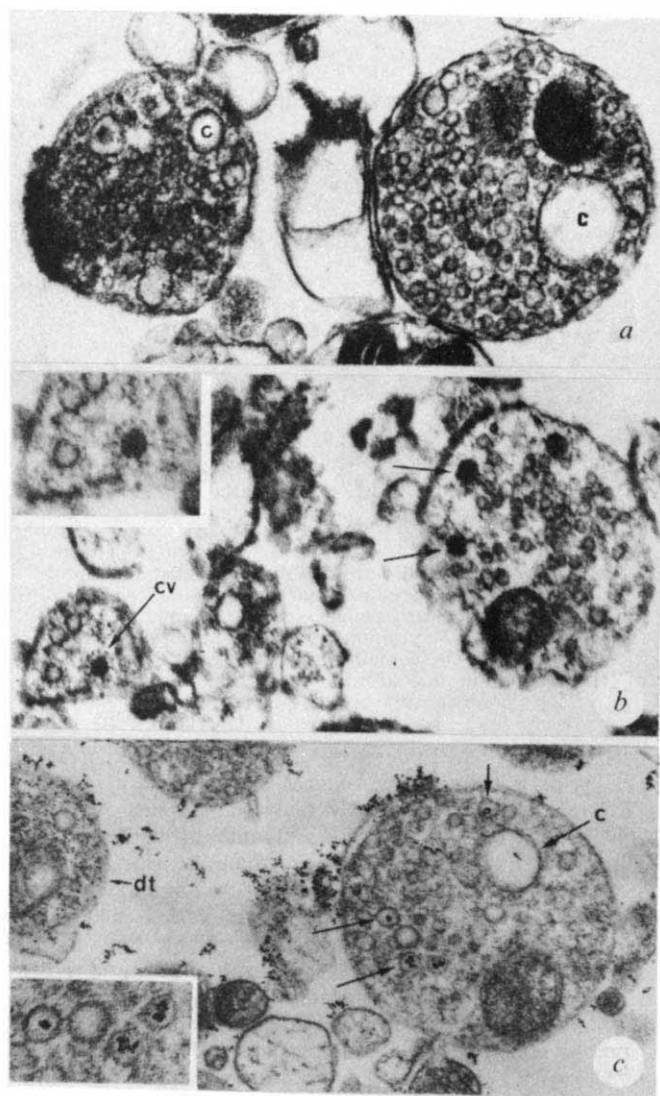


Fig. 2 Morphology of synaptosomes incubated in K-rich media. *a*, Synaptosomes incubated in 100 Na+50K solution, without extracellular marker for 15 min. Note marked heterogeneity of synaptic vesicle size and presence of large vacuoles (c). $\times 41,000$. *b*, Synaptosomes incubated in 100 Na+50K containing 5 mg ml⁻¹ HRP, for 30 min. Note HRP-labelled coated vesicle (cv); unlabelled arrows indicate two other HRP-labelled synaptic vesicles. $\times 45,000$. Inset: coated vesicle at $\times 71,000$. *c*, Synaptosomes incubated for 2 min in 115 Na+35K containing 3.3% Thorotrast. Unlabelled arrows indicate some of the Thorotrast-containing vesicles. A Thorotrast-containing large vacuole (c), and a damaged terminal (dt) with diffusely distributed Thorotrast are also seen. $\times 50,000$. Inset: higher magnification of Thorotrast-labelled vesicles. $\times 88,000$.

vesicles filled with electron-opaque material (presumably HRP reaction product) were seen in some of the terminals; this is illustrated in Fig. 2b, which also shows a coated vesicle filled with HRP reaction product. This latter finding fits with the hypothesis^{16,21} that one of the steps in the vesicle retrieval process may involve coated vesicles. Further evidence of synaptic vesicle recycling was obtained with Thorotrast-treated terminals, where both vesicles and large vacuoles contained the marker even after a short period of incubation in a depolarising solution²² (Fig. 2c).

We have repeatedly observed that the tracer-labelled vesicles (Fig. 2b and c) are usually larger than most vesicles in 'control' synaptosomes (Fig. 1). Preliminary evidence (unpublished) indicates that the initial step in membrane retrieval seems to involve the pinching-off of these large vesicles from the plasma membrane. The further implications of large vesicle formation are being investigated.

Our observations are consistent with a mechanism involving transient plasma membrane fusion and recycling of synaptic vesicles. They suggest that synaptosomes behave like intact terminals with respect to the mechanism by which they release vesicular contents. Thus, synaptosomes may be useful for the study of transmitter release and vesicle recycling processes in the central nervous system.

We thank J. A. Arndt, W. dePalma and T. McKenzie for assistance, Drs M. B. Bunge and Y. Fukami for guidance with the electron microscopy, and Drs N. C. Kendrick, D. Purves and C. M. Rovainen for suggestions regarding the manuscript. Supported by NIH. R. C. F. was a Grass Foundation undergraduate fellow.

ROBERT C. FRIED

MORDECAI P. BLAUSTEIN

Department of Physiology and Biophysics,
Washington University School of Medicine,
St Louis, Missouri 63110

Received February 9; accepted March 29, 1976.

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Extremely low frequency, weak electric fields affect schedule-controlled behaviour of monkeys

THE effect of weak electric fields on behaviour of organisms has potentially important implications for basic neurophysiology and evaluation of environmental hazards (chronic exposure to weak 60-Hz electric fields has become a way of life). We have demonstrated¹ that weak (approximately 7 V m⁻¹ p-p), extremely low frequency (7 Hz) electric fields could modify a lever-press response in monkeys. We summarise here data² confirming that finding for a larger group of animals with more replications and presenting evidence for a dose-dependent relationship for

7-Hz fields. In addition there are indications of a threshold of 10–56 V m⁻¹ p-p for field frequencies higher than electroencephalogram (EEG) range (1–32 Hz). Adey³ has hypothesised that since these field levels are clearly too low to trigger synapses in the fashion prescribed by classical neurophysiology, more subtle membrane sensitivities may be functioning as central nervous system mediators of the observed behavioural effects. One possible mediator is the degree of calcium efflux across the membrane⁴.

We have explored possible behavioural effects of fields at frequencies from 7–75 Hz and voltages from 1–56 V m⁻¹ p-p, together with a preliminary study at 100 V m⁻¹ p-p. Five female *Macaca nemestrina* monkeys (3–6 kg) were trained on a Skinner⁵ conditioning schedule. The animals had to wait 5 s between lever presses and to press within a 2.5 s 'limited hold'; each response recycled the 5-s delay period—an inter-response time (IRT) schedule (IRT > 5 s; < 7.5 s). The analysis of behaviour in terms of IRT has been widely used to evaluate behavioural effects of low doses of drugs (for example, ref. 6). For details, see Fig. 1, which shows an example of the histogram of IRTs across three voltage levels (1, 10 and 56 V m⁻¹ p-p) for the 7-Hz condition.

Our data analyses are limited to voltage levels of 1–56 V m⁻¹ p-p because preliminary data at the 100-V m⁻¹ level suggested that fields of this strength affected the randomly interspersed control days—that is, there seemed to be a 24-h 'carry-over' effect. Evaluation of possible effects at this level awaits further experimentation.

For fields with voltages from 1–56 V m⁻¹, the *a priori* hypothesis of shortened IRTs was tested with *t* tests (Fig. 2). Since our previous data¹ had indicated that low voltage (~7 V m⁻¹) low frequency (7-Hz) fields resulted in shorter times between responses than control no-field conditions, it was possible to examine the present data with this specific statistical hypothesis. At 1 V m⁻¹ none of the observed differences between field and no-field mean IRT ± s.d. was statistically significant. There is evidence for a frequency-specific (7-Hz) threshold at 10 V m⁻¹ p-p. The difference observed is small (~0.1 s) but significant at the 0.05 level. This low threshold for 7 Hz may reflect the fact that this frequency is in the range of normal brain wave activity (hippocampal theta) for performing monkeys. At 56 V m⁻¹, both

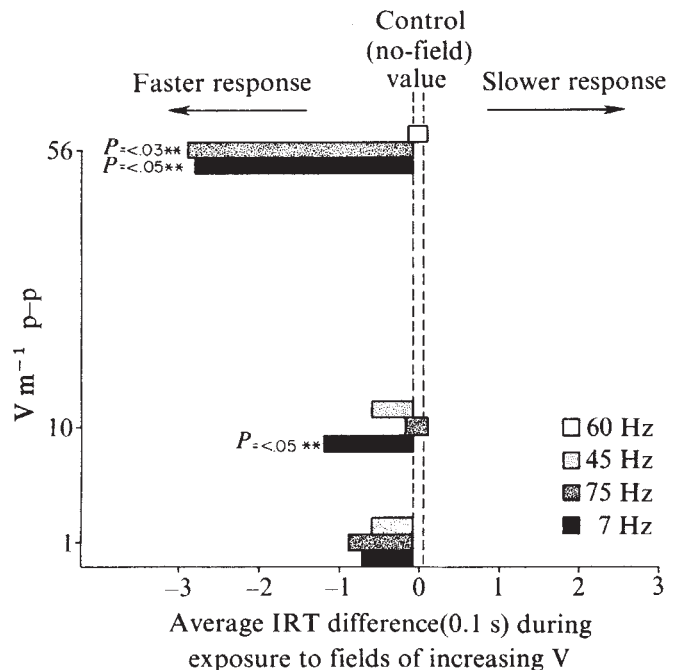
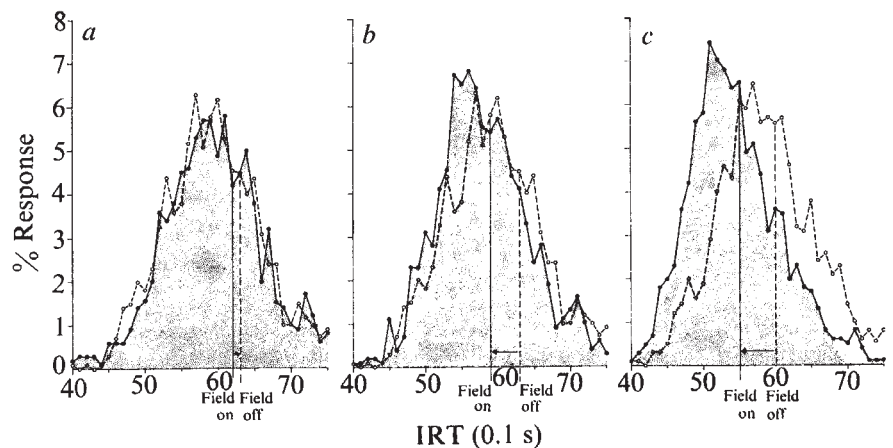


Fig. 2 Faster response (reduced IRT) as a function of frequency and voltage of applied electric field. Asterisks denote statistically significant shifts. All data are based on four repetitions of each experiment for each of 4–5 monkeys. Note the low voltage threshold (10 V m⁻¹) for 7 Hz, a frequency which is relevant to normal brain activity.

7-Hz and 75-Hz fields produce large (~0.3 s) and statistically significant reductions in IRT. Note that these data suggest a dose-dependent relationship for 7-Hz fields; mean IRT differences increase systematically across the three voltage levels from 0.07 s at 1 V m⁻¹ to 0.12 s at 10 V m⁻¹ to 0.29 s at 56 V m⁻¹.

These results substantiate our earlier finding that the 7-Hz field was associated with a very low voltage threshold of

Fig. 1 An example of the IRT distributions observed for one 4-h experiment for one unimplanted animal at 1 (a), 10 (b) and 56 V m⁻¹ p-p (c). Field frequency = 7 Hz. Exposure to this field resulted in a small shift towards faster IRTs at 10 V m⁻¹ and a much larger shift at 56 V m⁻¹. The average difference observed over all monkeys for this field configuration was 0.07 s at 1 V m⁻¹, 0.1 s at 10 V m⁻¹ and 0.3 s at 56 V m⁻¹. Field (●) and no-field (○) distributions are contrasted. Number of responses and $\bar{x}$ for field on: a, 1,024 and 62; b, 1,026 and 59; c, 973 and 55. Field off: a, 1,505 and 63; b, 1,505 and 63; c, 987 and 60. Histograms for both field and no-field conditions are a good approximation of the normal distribution. Four such histograms were generated for each frequency-voltage combination for each animal. About 300 field and no-field experiments were carried out. Correct responses were reinforced with small quantities of fruit juice; water deprivation was used during the initial stages of training but was not necessary during testing. No external cues (lights, bells) signalled availability of the reinforcer; the animal was required to respond to the lapse of time following the occurrence of its own preceding response. After 80–100 d training, stable performance (70–80% correct) was achieved and a random series of field/no-field tests carried out. Three animals were implanted with EEG electrodes and two were not, so that possible 'antenna' effects due to the presence of the electrodes could be assessed. Four 4-h replications of each field condition



and each control condition (no-field) were analysed for each monkey. Field conditions included frequencies of 0, 7, 45, 60 and 75 Hz, and voltage levels of 0, 1, 10, 56 and 100 V m⁻¹ p-p. One animal died from accidental causes at the conclusion of the 10 V m⁻¹ experiments so *n* was reduced to 4 for the experiments at 56 and 100 V m⁻¹. Experiments were carried out in a double-bronze mesh screened enclosure; a.c. conductors (except for a shielded camera) were excluded. Monkeys sat in plastic chairs between parallel field plates

(1 m² 50 cm apart) centred in the enclosure. The fields were measured with a specially devised fibre optic probe, and were found to be satisfactorily uniform and balanced⁷. Current from the imposed field flowing to ground through the implanted electrodes was measured in a phantom head at slightly less than 0.9 nA at 10 V m⁻¹ p-p. IRT was tape recorded and analysed by computer in 0.1-s intervals. Responses longer than 17.5 s were excluded to avoid skewing the distribution with occasional very slow responses.

Table 1 Summary of *t* tests for mean IRTs $\pm$ s.d. (no-field minus field differences, across voltage levels)

Voltage (V m ⁻¹ p-p)	Frequency (Hz)	No. of monkeys	Average no. of responses	Mean difference $\pm$ s.e.d. (10th s)	<i>P</i>	s.d. difference $\pm$ s.e.d. (10th s)	<i>P</i>
1	7	5	3,299	0.70 $\pm$ 0.69	< 0.20	0.12 $\pm$ 0.97	< 0.50
	45	5	4,229	0.55 $\pm$ 1.35	< 0.40	-0.28 $\pm$ 1.04	< 0.45
	75	5	3,360	0.94 $\pm$ 0.62	< 0.15	-0.20 $\pm$ 0.66	< 0.40
10	7	5	3,560	1.18 $\pm$ 0.48	< 0.05*	1.60 $\pm$ 0.52	< 0.03*
	45	5	3,982	0.55 $\pm$ 0.61	< 0.25	0.10 $\pm$ 0.41	< 0.45
	75	5	3,560	0.00 $\pm$ 0.60	—	-0.12 $\pm$ 1.06	< 0.50
56	7	4	2,764	2.88 $\pm$ 1.27	< 0.05*	1.78 $\pm$ 0.35	< 0.01*
	60	4	2,662	-0.03 $\pm$ 0.70	< 0.50	0.58 $\pm$ 0.28	< 0.10
	75	4	2,862	2.90 $\pm$ 0.72	< 0.03*	2.25 $\pm$ 0.69	< 0.03*

*Statistically significant shifts.

approximately the same level (7 V m⁻¹ previously and 10 V m⁻¹ here) and indicate much higher thresholds for frequencies which are not relevant to normal brain wave activity. For 75 Hz, the threshold is between 10 and 56 V m⁻¹; for 60 Hz it may be higher.

Numerical data and *t* tests for the mean IRT $\pm$ s.d. for voltage levels from 1–56 V m⁻¹ p-p are summarised in Table 1. No effect is observed at 1 V m⁻¹. At 10 V m⁻¹, the 7-Hz field was associated with a significantly smaller s.d. At 56 V m⁻¹ all field frequencies were associated with s.d.s smaller than the no-field values. These differences were statistically significant for 7 and 75 Hz, and reach a borderline level (*P* < 0.10) for 60 Hz.

Data for individual animals reflect the orderliness of the average values. At 1 V m⁻¹ the mean IRTs varied randomly across the field conditions. At 10 V m⁻¹, the 7-Hz condition was associated with the fastest mean IRT and smallest s.d. of all field conditions for every animal tested. At 56 V m⁻¹ all field-associated s.d.s were smaller than the no-field condition for every animal. All mean IRTs, except those associated with the 60-Hz field, were faster than no-field means at 56 V m⁻¹ for every animal tested.

Table 2 Analysis of variance of implanted compared with unimplanted animals at 1 and 10, 56 and 100 V m⁻¹

Mean IRTs $\pm$ s.d.							
Voltage Source		1 and 10 V m ⁻¹		56 V m ⁻¹		100 V m ⁻¹	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Group	$\bar{x}$	0.048	0.84	0.770	0.47	0.708	0.49
	σ	0.378	0.58	0.014	0.92	1.469	0.35
Replication	$\bar{x}$	0.953	0.46	0.769	0.55	0.497	0.70
	σ	0.412	0.75	0.865	0.51	2.434	0.16

Analysis of variance of IRTs for implanted compared with unimplanted animals revealed no significant effects (Table 2). Thus, it is improbable that the observed effects can be attributed to the presence of EEG recording electrodes in the brain (the so-called 'antenna' hypothesis). Additional analyses of variance were carried out for IRT data across frequency and voltage levels to look for possible information secondary to the initial effect of shortened IRTs. These analyses revealed no unexpected findings. Field exposure contributed significantly to the variance of the s.d.s of mean IRTs at low voltages (1 and 10 V m⁻¹ exposures, *F* = 2.59, *P* < 0.05), but not to the average IRTs—probably because of the small *n*. At 56 V m⁻¹ both s.d.s and average IRTs were associated with significant *F* values (*F* = 5.75, *P* < 0.02 and *F* = 5.22, *P* < 0.03, respectively). Replications did not contribute significantly to the variance at any of the voltage levels tested.

Ethological studies of electric and magnetic fields lend support to the behavioural data presented here. Keeton⁸ demonstrated that weak magnetic fields can affect the homing of pigeons; certain species of fish utilise weak electric fields to detect their

prey⁹; and 10-Hz electric fields (25 mV cm⁻¹) can alter circadian rhythms of activity in man¹⁰. Laboratory studies to date have provided only equivocal evidence^{11–15}. Absence of a clear-cut picture may be related to specific attributes of the separate experiments in such factors as field frequency, voltage, duration of exposure and behavioural assay. Keeton's pigeons ignored the presence of an imposed magnetic field if the Sun were available as a homing cue. Similarly, weak electric fields may not affect performance on demanding behavioural tasks with multiple stimuli inherent in them. An animal carrying out such a task may be able to 'ignore' the presence of the field.

On the basis of studies on calcium binding in cerebral tissues^{16,17}, which have paralleled the present studies on behavioural effects of weak electric fields, and taking into consideration the physical model conceived by Grodsky¹⁸, we propose that the potential for sensitivity to weak electric fields exists in all organisms whose nervous system is characterised by: slow electrical waves in the extracellular space; and a system of macromolecular sensors on the membrane surface which is capable of transducing components of the extracellular field by altered ion binding and an associated conformational change. In animals in which field detection is not intimately linked to survival, sensitivity can probably only be revealed in innocuous experiments of the kind described above which are characterised by long exposure duration, little external stimulation and low motivational requirements.

We thank the Naval Electronics System Command, Office of Naval Research and the Bureau of Radiological Health, Washington, DC, for support; and J. Frane of UCLA Health Sciences Computing Center for advice on the statistical analyses.

R. GAVALAS-MEDICI
S. R. DAY-MAGDALENO

Brain Research Institute,
Center for Health Sciences,
University of California,
Los Angeles, California 90024

Received August 8, 1975; accepted March 1, 1976.

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Antivenom activity of rattlesnake blood plasma

MANY species of venomous snakes have a high resistance to their own venoms and to those from other individuals of the same species and other species^{1–4}. The resistance is not absolute but it increases as the size of the snake increases, and varies also with the type of venom. Blood plasma or sera of several species neutralise the venom of the same species and of other species both *in vivo* and *in vitro*^{5–11}. As Clark and Voris¹⁰ reported, the whole serum of the eastern diamondback rattlesnake (*Crotalus adamanteus*) neutralises lethal doses of *C. adamanteus* venom in mice and the protective capacity of the serum is associated with the serum albumin fraction. Mixtures of *C. adamanteus* serum and venom show no evidence of precipitation, such as occurs when serum from animals hyperimmunised with *C. adamanteus* venom is mixed with *C. adamanteus* venom. The nature of the factor(s) in rattlesnake plasma, which neutralise rattlesnake venom toxins, and the mechanism of neutralisation are unknown. We have found that both western and eastern diamondback rattlesnake (*C. atrox* and *C. adamanteus*) blood plasma contain antivenom factor(s) and have a neutralisation capacity (NC), not only for *C. atrox* venom but also other rattlesnake venoms. The antivenom activity of *C. atrox* plasma is sufficiently potent, compared with commercial immunoglobulin antivenin, to be potentially useful in the management of snakebite.

Before testing for *C. atrox* plasma antivenom activity, we heated the plasma at 56 °C for 30 min to detoxify it¹². The intraperitoneal toxicity (LD₅₀) of unheated *C. atrox* plasma in mice varied from 2.6 mg per 20 g mouse to 9.0 mg per 20 g mouse for plasma from 25 individual specimens of *C. atrox*. Pooled plasma (LD₅₀, 5 mg per 20 g mouse) from 100 captive *C. atrox* was used. Heating removed the toxicity (LD₅₀>40 mg per 20 g mouse) and all subsequent experiments were carried out with heat-treated plasma. Biuret¹³ assay was used to estimate protein content of plasmas and venoms and showed a protein content of about

40 mg ml⁻¹ or about 50 mg ml⁻¹, when corrected for dilution by citrates for pooled *C. atrox* plasma. All antivenom and toxicity assays were done on a protein weight basis.

We found that both *C. adamanteus* and *C. atrox* plasma were potent antivenoms for several rattlesnake venoms (Table 1). The neutralisation capacity of both plasmas was compared with that of commercial antivenin by injecting mice with 5 mg of plasma protein or antivenin intraperitoneally and determining the LD₅₀ of the venom in the antivenom-treated mice compared with that of untreated mice. Both plasmas had significantly higher neutralisation capacity values for various rattlesnake venoms than did antivenin, except in the case of South American rattlesnake venom (*C. durisus terrificus*) and the mohave rattlesnake venom (*C. scutulatus scutulatus*, brown variety). We found two varieties of *C. scutulatus scutulatus* readily distinguished by the toxicity of their venoms and the capacity of antivenoms to neutralise each venom. The two varieties are distinguished also by their colour. The yellow variety has more toxic venom and lower neutralisation capacity values for antivenoms than the brown variety.

Rattlesnake venoms are neutralised also when premixed *in vitro* with either rattlesnake plasma or commercial antivenin. A visible precipitate occurs with immunoglobulin antivenin but not with plasma.

To test the hypothesis that the antivenom activity is due to antigenic protein or multiple proteins in *C. atrox* plasma, rabbits (New Zealand White) were injected with heat-treated *C. atrox* plasma and Freund's complete adjuvant and the resultant antisera were collected 1 week after a booster injection of *C. atrox* plasma and adjuvant 4–6 weeks after the initial injection. The antivenom activity of *C. atrox* plasma was removed completely when a 5-ml sample was treated three times with 1 ml of antisera and the resultant precipitates were removed by centrifugation. Addition of unimmunised rabbit serum to *C. atrox* plasma did not diminish the antivenom activity.

Clark¹⁰ found the antivenom activity of *C. atrox* serum in the albumin fraction after chromatography on Sephadex G-100. Our results of polyacrylamide gel electrophoresis (PAGE)-immunodiffusion studies indicate that the antivenom activity is due not to albumin or some small molecule bound to albumin, but to separate antigenic proteins in the plasma.

Plasma proteins were fractionated on gradient (4.5–8% acrylamide) gel slabs using the discontinuous basic buffer system described by Ortec Inc. Each slab was cut into three sections and minced; the protein was extracted into saline and filtered through a 1-μm Millipore filter. Extract from each gel section was assayed for antivenom activity. The

Table 1 Capacity of four antivenoms to neutralise lethal toxicity of rattlesnake venoms

Venom	Pig plasma	Neutralisation capacity		
		Antivenin	<i>C. adamanteus</i> plasma	<i>C. atrox</i> plasma
<i>C. adamanteus</i>	1.3	4.5	7.7	6.5
<i>C. atrox</i>	1.4	2.7	3.5	4.3
<i>C. viridis concolor</i>	1.3	6.4	9.8	9.0
<i>C. durisus terrificus</i>	1.1	9.5	8.1	8.3
<i>C. scutulatus scutulatus</i> , brown	1.3	10.2	9.0	10.1
<i>C. scutulatus scutulatus</i> , yellow	1.1	2.7	5.8	6.4
<i>Naja naja kaouthia</i>	1.1	1.0	1.0	1.0

Venom lethal toxicity (LD₅₀) was assayed using Swiss-Webster mice (18–20 g). Twenty to thirty mice were used for each assay and the LD₅₀ dose was calculated according to the method of moving average interpolation^{14–16}. Venoms from rattlesnakes and cobras were obtained from our captive specimens by mild electrical stimulation of the mandibular compressor glandulae muscles surrounding the venom gland¹⁷. Venoms from several healthy individuals, strictly of the same species, were pooled and centrifuged to remove cellular debris, then lyophilised and stored at –20 °C as a batch. Venoms handled in this manner can be stored for years without measurable loss of lethal toxicity. Neutralisation capacity is the ratio of the intraperitoneal LD₅₀ of venom in mice treated with antivenom (5 mg protein given intraperitoneally) to the intraperitoneal LD₅₀ of venom in untreated or saline-injected mice. A neutralisation capacity of 1 means that the antivenom had no effect on the LD₅₀ of venom in mice in 24 h. The greater the neutralisation capacity the more potent was the antivenom. Pig plasma was obtained from a local slaughterhouse from blood collected in trisodium citrate (3.8%, 4 parts blood: 1 part citrate). Antivenin was Crotalidae polyvalent, equine, North and South American antisnakebite serum (Wyeth). *C. atrox* and *C. adamanteus* plasma were obtained from specimens in our serpentarium, either by heart puncture under anaesthesia¹⁸ or by decapitation. The plasma was separated from individual citrated bloods and then pooled, centrifuged and stored frozen (–20 °C). Plasma was taken and stored in this manner to avoid serum clots. The antivenom activity of plasma and serum was the same.

Table 2 Effectiveness of *C. atrox* plasma compared with antivenin on mean survival times of New Zealand White rabbits (~2.7 kg) injected with *C. atrox* venom (6 mg kg⁻¹)

Treatment	Antivenom pretreatment time (h)							
	-12	0	24	48	72	96	120	144
None	22±4	22±4	—	—	—	—	—	—
Pig plasma (1,000 mg)	20±4	22±4	22±4	—	—	—	—	—
Antivenin (200 mg)	24±4	30±4	24±4	—	—	—	—	—
<i>C. atrox</i> plasma (heated) (200 mg)	24±4	S	32±4	22±4	—	—	—	—
Antivenin (400 mg)	26±4	50±4	22±4	—	—	—	—	—
<i>C. atrox</i> plasma (heated) (400 mg)	26±4	S	45±5	28±4	22±4	—	—	—
Antivenin (800 mg)	26±4	S	22±4	—	—	—	—	—
<i>C. atrox</i> plasma (heated) (800 mg)	26±4	S	S	S	S	S	50±8	25±5

Each group consisted of three rabbits. Antivenom was injected intravenously either at the same time as the venom (intramuscularly) (that is pretreatment time = 0) or before the injection of venom at several pretreatment times (24–144 h). The first column minus 12 h (–12) refers to injection of venom 12 h before injection of antivenom. S indicates that all animals survived for several weeks until they were killed. Survival times are in hours.

activity was found in the middle gel cut separate from albumin. Analytical PAGE of the extract revealed four protein staining (Coomassie blue) bands 1.5–2.5 cm behind the albumin band. This group of proteins also stained strongly for carbohydrate.

To determine whether the antivenom activity of *C. atrox* plasma is potent enough to be of potential value in snakebite therapy, we compared its effectiveness with that of antivenin. The plasma was more effective in increasing survival time than any dose of antivenin (Table 2). This is remarkable as whole *C. atrox* plasma was used and, as shown by PAGE, only a small amount of the total plasma protein has antivenom activity. In contrast antivenin is a purified horse serum immunoglobulin preparation. Thus *C. atrox* plasma is a very effective antivenom.

We also found that *C. atrox* plasma activity against *C. atrox* venom was maintained for up to 4 d *in vivo* (Table 2), whereas antivenin rapidly loses its effectiveness (6–12 h) *in vivo* (rabbits).

We found the same antivenom pretreatment effect in mice by determining neutralisation capacity against *C. durisus terrificus* venom as a function of pretreatment time. There was a steady decline in neutralisation capacity during 3 d after a single intraperitoneal injection of antivenom (10 mg protein). The curve is somewhat steeper for *C. durisus terrificus* venom compared with that of *C. atrox*.

In these experiments, treated rabbits and mice showed markedly less tissue damage and haemorrhage than untreated animals. Isolation and characterisation of the antilethal factor(s) in *C. atrox* and *C. adamanteus* plasma may show that it is similar to the antihæmorrhagic factor found in Habu (*Trimeresurus flavoviridis*) venom reported by Omori-Satoh¹¹.

Even though immunoglobulin antivenin, prepared from sera of horses immunised against venom, is the most effective agent used in the management of snakebite, there are many serious problems associated with its use; for example, allergic reactions and the length of time from the bite to the effective administration of antivenin. Perhaps an antivenom prepared from snake plasma or a synthetic product based on antivenom factor(s) in snake plasma would be useful and safe for both the first aid and medical management of snakebite.

We thank the Charles Connors family, Broken Bow, Oklahoma and the Junior Chamber of Commerce, Okeene, Oklahoma, for health specimens of *C. atrox*, and Mr Don Bennett, Oklahoma City, for *C. atrox* plasma. We also thank Mr Phil Garn and Mr Robert Larsen for assistance.

R. STRAIGHT
J. L. GLENN

Research Service, 151J,
Veterans Administration Hospital,
Salt Lake City, Utah 84113

C. C. SNYDER

Department of Surgery,
College of Medicine,
University of Utah,
Salt Lake City, Utah 84112

Received February 10; accepted April 6, 1976.

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Erratum

In the article "Calcium activation produces a characteristic response to stretch in both skeletal and cardiac muscle" by R. L. Moss, M. R. Sollins and F. J. Julian (*Nature*, **260**, 619; 1976) there were the following errors: on p. 619, line 16 should have read . . . able to interact with thin filament sites . . . and not as printed. On p. 620, second column, line 27 should have read . . . cium (pCa about 9). Little if any short range elastic response . . . and not as printed.

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reviews

Symbionts or parasites?

Fred Dainton

The Force of Knowledge: The Scientific Dimension of Society. By John Ziman. Pp. ix+374, (Cambridge University: Cambridge and London, April 1976.) £7.50.

FRANCIS BACON firmly believed that natural philosophy could be organised for the benefit of mankind and no doubt good rational men holding power in an authoritarian state might entertain this hope. But both science and society change and so do their interactions. In his book, which arrived on the 350th anniversary of Bacon's death, Professor Ziman traces the changes in this relationship in the period from Bacon to the present day.

It is difficult to encapsulate the author's thesis in a few phrases but he seemed to be saying that knowledge—that is, science—is only useful if it leads to the creation of new knowledge or the solution of social problems or the acquisition by a society of physical power over resources and energy; and for all these purposes it must be communicated between men. But new ideas came from the sifting, re-arranging and assessing sceptically facts and earlier concepts in the minds of individuals. So in the first few chapters we are given glimpses of individual scientists, their styles of work, how their institutions for discourse and intercourse developed from clubs to learned societies and professional associations in response primarily to the increasing need (and difficulty) to communicate.

His characters are skilfully chosen and his vignettes are beautifully drawn: there is the restless, questing intellect of Leonardo; the convergent, introvert Cavendish drawing solitary satisfaction from precise observation and measurement; Ramanujan, the untutored genius; Wegener the inspired advocate of continental drift admitting only the evidence favourable to his belief; the courageous, pugnacious, rational, iconoclastic ideologue J. B. S. Haldane and the brilliant but conservative all-rounder Fermi forced by the social significance of the work he initiated to face up to his social responsibility. All this and much else besides is offered to the reader in felicitous evocative prose accompanied by beautiful illustrations; but I found it curious that such an author should stress the cogni-



Einstein—personal authority accorded by his own republic of learning

tive at the expense of the affective in his subjects. Thus we read nothing of their frequent passion for music and mountains. From the intercommunication of scientists it is a short step to their communication with laymen where, oddly, the roles of the Extra-Mural Departments of Universities, the Open University, of the Literary and Philosophical societies and other mutual improvement movements receive no mention.

Professor Ziman is at his best in showing us how personal authority whether accorded by the state—for example, as to Lindemann and Oppenheimer—or by their own republic of learning—for example, as to Darwin and Einstein—is in the long run the enemy of progress in science. Healthy science requires the maintenance of a vigorous scepticism in its practitioners, who should not be obedient priests but democratic and critical republicans. But alas scientists are human too and the revolutionary of yesteryear tilting at authority is easily turned by success

into the established figure of today resisting the attacks on his ideas, position and influence. After a brief glance at medicine—taken principally, one feels, to illustrate the fundamentally reactionary nature of craft guilds and trade unions exerting powers they have wrested from rather than been willingly awarded by society—the reader moves to stories of industrial innovation (zips, jet engines, nylon, penicillin, float batteries) which the author believes to be “one of the dominant social processes of our time”. It may well dominate less in future as such innovation makes further inroads on our resources of energy and raw materials; an aspect which like pollution (*pace* a brief reference on p279) is one of the outcomes of ‘science in action’ which is completely disregarded.

By now (chapter 9) the reader has been brought to the third quarter of the twentieth century, the scientist is transmogrified from the independent researcher to the more or less willing member of a large team, completely institutionalised, serving the military or civil ends of the state or the commercial ends of a corporation or ‘big science’; or, if he is ‘academicised’, behaving in the ritual research fashion to enliven his teaching and to earn the approval of his senior colleagues which he esteems for its own sake and/or because it aids his promotion. Next follows a look at the transplantation of science from Western to alien soil in Australia, Japan, India and in that hypothetical but so clearly recognisable and authentic newly emerged Republic of Saturnia and the tragi-comic outcome of this dislocation. We proceed to a child's guide to the social sciences where controlled experiments are impossible, all systems are multivariate (and not independent variables at that!) and people still persist in the exercise of ‘free will’, the greatest ‘joker’ in the pack. So by way of the rarely felt moral dilemma of scientists, employed in increasing numbers in the war game, we come to the final chapter which is really an essay in the necessity of scepticism of authority. True as this is, it hardly seems to get to the roots of the scientists' social responsibility and it is a pity that Professor Ziman did not allow the current of his fluent prose to carry him to some deeper commitment.

This said we must remind ourselves that the book is the result of his preparing himself, as his conscience dictated he must, "to introduce the theme of the social relations of science and technology" to the average second year university student of natural science. In this aim he has succeeded brilliantly. Such students with 'A' levels in physics and chemistry should be able fully to understand the scientific, technological and engineering content. Moreover, based either on my own recollections of myself 40-odd years ago or on conversations with present-day students I am convinced that the style, topics and sentiments displayed in the book should have a strong appeal to the sense and sensibilities of young adults. They should find it as fascinating as a good novel, replete with characters whose individual and collective attributes are mirrored in many of their seniors and who face issues of great contemporary relevance and importance. I hope that they will put it down with the feeling that their generation has to meet the challenge of how the scientist is to be the critical symbiont of the larger society of which he is a part, helping to shape its purposes rather than becoming either the parasite or the passive helot of a society to whose aims he is indifferent if not hostile.

There is yet another reason why, were I still a professor, I would strongly recommend this book to my students and more particularly to their non-scientific contemporaries. It is simply that the book conveys in a lucid and lively manner the essence of the attitudes of scientists and by marked contrasts of style and matter jolts the reader into new thoughts. Thus it is serious in intent but impish in style, reverent and irreverent by turn, and popular—almost shallow treatment of some matters nevertheless often contain implicit or explicit insights of some depth. They serve to give the book a touch of human frailty seeming to put the author on the same level as the readers and thus encouraging them to think critically rather than be the passive receptors of the dispensed, predigested pabulum of scientific authority proffered to them in the textbooks. But scholars will sigh over inaccuracies and over-simplifications or exaggerated phrases, and twice I narrowly escaped apoplexy. No reader will be indifferent and the young will be stimulated as the author wanted them to be. All that remains to be asked for is a paper back at half the price of the hard copy.

Sir Frederick Dainton is chairman of the University Grants Committee of the UK.

Unexpected treasures

The Compacted States of Vitreous Silica. By William Primak (Studies in Radiation Effects in Solids, Vol. 4.) Pp. xv+184. (Gordon and Breach: New York, London and Paris, 1975.) £10.70.

CONSIDERING the terrestrial abundance of silica and the crucial position it holds in several fields of technology, our understanding of its structure and solid-state chemistry is deplorably vague. An excuse can be given—that the solid states of SiO_2 are many and complex and have frustrated many conventional attacks—but the response of science has been lax. Pure scientists, instead of mounting more unconventional attacks, can be accused of generally turning away from the problems and letting the technologist flounder. A short list of technological uses of silica includes transmitting and reflecting optics, ceramics, piezoelectric devices, crucibles, passivating and insulating films; some unusual thin-film cantilever structures are now being used for displays and other purposes. In many of these applications, vitreous silica is used and yet, because conventional crystallography cannot work on such amorphous structures,

ignorance of this state of the material is the greatest.

Primak's book-length review article presents a highly individual view of the structure of this amorphous material, based on physical measurements which can certainly be classed as unconventional but were superbly adapted to the problem. Compaction is an effect which occurs only in non-crystalline solid networks (perhaps even only in oxide networks but Primak omits to tell us if any non-oxide glasses will compact); it is produced by pressure-cycling, high temperature, shock waves or radiation and can so alter the vitreous silica network that density is permanently reduced as much as 15% for pressure, and 3% for the others. Alkali silicate glasses will compact even more. This behaviour is so foreign to our concepts of immutable lattice constants that it is scientifically important; technological uses are, of course, also conceivable. Primak's own work, mainly on radiation-induced effects, is a classic of well-performed experimental study, augmented by careful but not hide-bound interpretations. His hypothesis for the compaction was startling in that it went clean against the instinctive feeling of other workers that an increase in density implied an increase in order (fused silica is less dense than quartz).

He supplies ample evidence that silica can reduce its volume by the formation of a 'kink' in the large (say twelve-atom) rings of the silica network so that a 'void space' which was previously empty is newly occupied and order thereby decreased. But why should the kink be retained? The wider, new idea comes in the proposal that the silica network is in "a microscopic state of balanced stress" and that an excitation, producing one kink, causes the whole local structure to rearrange, perhaps in the form of a "network segment" which re-configures its quota of non-bridging oxygen atoms. Although Primak does not elaborate the model with any theoretical solid-state physics, it is still a very meaty and useful hypothesis. The book is a pursuit of its proof through every scrap of evidence available (there are over 350 references). Although the book was conceived nine years ago and largely finished six years ago, the central hypothesis and the suggestions for further investigations remain valid. Appendices embrace new research up to 1973.

Only occasionally does the relaxed personal style lead to vagueness; for example, the author never tells us the best estimate for the Si-O-Si bond angles and lengths in the seven main phases of silica, and this sort of thing does detract from the introductory value. Later chapters are packed with intense and creative reasoning. Parochialism sometimes shows up here—for example in the lack of use which Primak has made of the electronic-optical disciplines of solid-state physics—for example, he does not explain the relevance of infrared spectra, or more than quote a few measurements. His only definite error in the electronic field is to say that "vitreous silica has no proper band structure", a common but unfortunate misconception, disproved by Di Stefano and others; the field of colour centres is dismissed as a "morass" and, although work on thin silica films is mentioned in passing, he has failed to note a valiant attempt made in 1969 by H. L. Hughes to explain γ -ray-induced surface-state generation in such films, using a compaction model correlating his and Primak's data. One feels that great benefit would have been bestowed on the world if the author had wandered a bit further in these fields.

The book does give people interested in silica a chance to revel in a refreshing minor tributary of knowledge which may yet do great things for the mainstream. By his pleasant but creative style the author indicates some of the unexplored creeks which could yield unexpected treasures from the most abundant material in the earth's crust.

Andrew Holmes-Siedle

Aphids

Aphids. (Invertebrate Series.) By Roger Blackman. Pp. 175. (Ginn: London and Aylesbury, 1975.) £3.00.

THE main reason why aphids have not attracted the attention they merit is the lack of simple accounts of their biology and taxonomy. Roger Blackman in 175 pages presents such a comprehensive, highly readable and extremely well illustrated account of aphids. The book's main asset is Chapter 10, 'Aphids in Britain'. This chapter makes available to amateur entomologists, for the first time, a workable key in English to the more common genera of aphids in Britain. The excellent colour plates by Hilary Burn, which illustrate this chapter, serve to bring some of the aphids to life. Unfortunately the colour photographs in plate 1 are so badly reproduced that they are useless; an additional colour plate would have been preferable.

The other 10 chapters deal with the structure and physiology of aphids, natural enemies, relationship with ants,

population dynamics and techniques of study. This last chapter is a useful source of ideas for student projects.

In future editions Fig. 3 could be omitted, or revised, as it confuses rather than clarifies the discussion of an aphid's powers of multiplication; and Fig. 33 modified, as not all host-alternating aphids can produce and mature sexual morphs on their secondary hosts. The complex terminology of the various morphs of aphids has deterred many biologists from studying aphids, and it is therefore surprising that Dr Blackman did not use the simpler terminology proposed by Hille Ris Lambers (*A. Rev. Ent.*, **11**, 47-78, 1966). Unqualified statements such as aphids produce large numbers of offspring to counteract the losses they incur (p34), and that crowding is self induced (p78) unfortunately support Janzen's (*Science*, **182**, 1125-1126, 1973) contention that aphid biology is in need of a solid dose of evolutionary biology. Apart from these criticisms this book can be warmly recommended as an excellent introduction to a fascinating group of insects.

A. F. G. Dixon

Controversial texts

Techniques of Applied Quantum Mechanics. By J. P. Killingbeck. Pp. 224. (Butterworth: London and Boston, Massachusetts, November 1975.) £9.

OF the five chapters in this book two are directly relevant to the title—chapter four on "Perturbation and Variational Methods" and chapter five on "Group Theory", both topics in which the author has had practical experience. It is a pity that chapter four did not begin at the beginning—clearly a reader is supposed to have already read a standard book on quantum mechanics including the section on perturbation theory. The book would have been better if these chapters had been preceded by a conventional description of the principles of quantum mechanics. Instead we have a chapter called "Review of Basic Principles", which makes derogatory remarks on other textbooks, gives instructions to teachers of quantum mechanics and tells us about dyadics, but fails to review the basic principles. It is too nebulous in intent to be useful and is inconsistent in what is required of the reader—for example, if he needs to be told the meaning of operators commuting he should not be expected to be familiar with Dirac bra/ket notation. We then have two chapters on "Modern Mathematics" and "Operator Theory". In fact the mathematics of the second chapter is not in any sense modern. As a summary of some, mainly algebraic, topics these two chapters are adequate

and probably useful. They are, however, essentially a list of definitions and theorems. Little is actually done with the concepts discussed and there are no proofs. It is hard to see how any student can really learn a subject from this type of treatment.

When the author is writing about "Applied Quantum Mechanics" he writes well and it should be said that the relevant two chapters constitute more than half of the book. In other places he is less convincing and one detects a certain 'propaganda' aspect to the book, inappropriate in a textbook. On p10: "One of the basic problems is that of communication between the different types of scientist working on the subject; many a physicist has tried to follow up some interesting point which he suspects may have a practical application, only to give up in frustration at the large number of unfamiliar theorems and erudite subtleties which are taken for granted by the average mathematician's writings on quantum mechanics". Presumably it is "lack of communication" not "communication" that is alleged to be a problem. But is it a problem? What is the evidence?

On p38 we are told "The embedding of the real numbers in the complex numbers which is involved in this calculation is usually not commented on at all in texts concerned with quantum mechanics". Presumably these textbooks, contrary to the one under review, also omit to tell us that $\pm i$ is "more exactly $0 \pm li$ ". Maybe their authors know something! E. J. Squires

More to coral reefs . . .

Biology and Geology of Coral Reefs. Vol. 3 (Biology 2). Edited by O. A. Jones and R. Endean. Pp. xxi+435. (Academic: New York, San Francisco and London, 1976.) \$49.00; £25.00.

FOR the second biological volume of this composite work on coral reefs, the editors have assembled a highly competent team of collaborators. There are chapters dealing with the more important groups of associated organisms and others covering aspects of the ecology of both reefs and coral cays. (For a review of volumes 1 and 2, see *Nature*, **248**, 809, 1974.)

From the unique wealth of his long experience, F. R. Fosberg discusses coral island vegetation which, in its limited but widely distributed species, gives, as he concludes, an insight into the nature and complexity of vegetation generally, while itself forming the basis of the rightly famed beauty of coral islands.

A. J. Bruce reviews our present knowledge (to which he has contributed so extensively) of the numerous shrimps and prawns around coral reefs. Aspects of the ecology of coral reef fishes are similarly discussed by B. Goldman and Frank Talbot, again on the basis of much personal experience on One Tree Island in the Capricorn Group. A further chapter deals with the natural toxicity of certain coral reef fishes.

The highly vexed problem of ciguatera is the province here of its major investigator, A. H. Banner. This disease, which may unpredictably follow human consumption of coral reef fishes in the Indo-Pacific and the Caribbean, forms a major hazard in the developments of fisheries. We know the nature of the toxin but not the conditions controlling its appearance.

The factors responsible for the destruction and recovery of reefs are very suitably reviewed by R. Endean; there are further chapters on echinoderms, on intimate associates of reef corals and on birds and on turtles, the last from Robert Bustard who has contributed so much to our knowledge of those around the Great Barrier Reef.

Altogether this is an exceptionally valuable book to be studied and, if possible, possessed by the growing numbers of those fascinated by the innumerable problems presented by a living coral reef.

C. M. Yonge

obituary

Robert Franklin Mehl, Professor-Emeritus of Metallurgy at Carnegie-Mellon University, who died in Pittsburgh on January 29 at the age of 77 was undoubtedly one of the greatest academic metallurgists of the American scene. After studying at Franklin and Marshall College, Princeton and Harvard Universities, he went to the Naval Research Laboratory in 1927 as Director of the newly formed physical metallurgy laboratory. In 1932, after a brief period in industry, he became the first Director of the Metals Research Laboratory at the then Carnegie Institute of Technology, and launched on what was to be his greatest achievement. He collected around him an extremely talented group of physicists, chemists and metallurgists who in the next decade were to make outstanding contributions to physical metallurgy, particularly in the fields of diffusion, phase transformations and precipitation hardening. But perhaps Mehl's greatest contributions were towards a basic understanding of the various reactions involved in the heat treatment of steels, in particular the formation of ferrite from austenite and the pearlite and bainite reactions which he and his colleagues analysed so

thoroughly that they elucidated many of the principles from which the physical metallurgy of steels has developed.

In 1935 Mehl also became Chairman of the Department of Metallurgy, instituting scientifically based courses of high standard which in a few years made Carnegie Tech. a premier place for the study of metallurgy which had a large impact not only on the local steel industry but also throughout the United States. During World War II his laboratory was thus well placed to carry out work which greatly aided the production of guns and armour, though Mehl was often frustrated by reactionary attitudes in industry towards scientific endeavour!

After the war, he continued to stimulate the activities of the Metals Research Laboratory over an extremely wide range until 1960. Possessed of a very strong personality, he wielded his influence far and wide; inevitably his scientific contributions were now made with a broad brush rather than a rapier; however, the rapier was always ready at hand to deal decisively with anyone who might venture to take an opposite viewpoint. Numerous names of scientists in the MRL during the period 1945-60 now adorn

the faculties of many well known departments of metallurgy and leading industrial laboratories, a constant reminder of the high standards on which Mehl insisted.

He became a familiar figure in Europe between 1960 and 1966 when he was European consultant to the US Steel Corporation. The combined role of ambassador and interpreter of the European metallurgical scene suited Mehl at this stage of his life, when he was already one of the grand old men of metallurgy; he returned, however, to the USA in 1966 to become visiting professor at the University of Delaware and later at Syracuse University. Latterly, retired in Pittsburgh very close to his beloved University, he suffered severe ill-health, but set a fine example by his great courage, and by maintaining an active interest in his subject to the last. A paper he wrote in 1975 for the Annual Review of Materials Science is an excellent summary of his scientific career and is spiced with his own unmistakeable philosophy. It is indeed a fitting epitaph.

R. W. K. Honeycombe

Appointments

Dr Mart de Groot as Director of the Armagh Observatory.

Dr Thomas Anthony Connors as Director of the MRC Toxicology Unit.

Dr Peter Cardwell Elmes as Director of the MRC Pneumoconiosis Unit.

Dr R. R. Dils as Professor of Physiology and Biochemistry at the University of Reading.

Dr A. F. G. Wyatt as Professor of Physics at the University of Exeter.

Announcements

May 27-28, **Biology of Chemical Carcinogenesis**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).
June 3, **Contributions of Protozoa to Genetical Knowledge**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

June 17-18, **Quantitative Mass Spectrometry in Life Sciences**, Ghent (Prof A. De Leenheer, Laboratoria voor

Medische Biochemie en voor Klinische Analyse, Academisch Ziekenhuis, Rijksuniversiteit Ghent, 135 De Pintelaan, B-9000 Ghent, Belgium).

June 22, **Reclamation and Recycling of Metals**, London (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20 9LW).

June 26, **Educational Technology for the Handicapped Child**, Birmingham (P. R. Davie, Prospect Hall, Selly Oak Colleges, Birmingham B29 6LE).

October 16-20, **Molecular Basis of Host-Virus Interaction**, Varanasi, India (Dr M. Chakravorty, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India).

October 18-21, **Annual Meeting of the Association of Official Analytical Chemists**, Washington DC (L. G. Ensinger, Executive Secretary AOAC, Box 540, Benjamin Franklin Station, Washington, DC 20044).

November 17-19, **ACTH and Related Peptides: Regulation, Action and Structure**, New York (Conference Department, The New York Academy

of Sciences, 2 East 63rd Street, New York, New York 10021).

Erratum

In last week's **Appointments** section the new Professor of Botany at Royal Holloway College was incorrectly given as Dr J. D. Hodge. It should have been Dr J. D. Dodge.

Reports and publications

Great Britain

Family Planning in Five Continents: Africa/America/Asia/Europe/Oceania. Pp. 28. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, W1, 1975.) 65p; \$1.45 plus postage.

Bulletin of the British Museum (Natural History). Geology. Vol. 27, No. 1: A Reappraisal of *Prophaethon shubsolei* Andrews (Aves). By C. J. O. Harrison and C. A. Walker. Pp. 1-30 + 3 plates. (London: British Museum (Natural History), 1976.) £2.85.

Mineral Resources Consultative Committee. Mineral Dossier No. 15: Mica. Compiled by R. N. Crockett. Pp. v + 22. (London: HMSO, 1975.) 85p net.

Department of the Environment. Register of Research 1975. Part 4: Environmental Pollution. Pp. v + 258. (London Headquarters Library, Department of the Environment, 2 Marsham Street, SW1, 1975.) [102]
Ministry of Agriculture, Fisheries and Food. Agricultural Chemicals Approval Scheme: Insecticides/Fungicides/Herbicides. 1976 List of Approved Products and their Uses for Farmers and Growers. Pp. 190. (Hatching Green, Harpenden: Agricultural Chemicals Approval Organization, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, 1975.) [112]